Rodent-borne *Leptospira* spp. and hantaviruses in Europe

Inauguraldissertation

zur

Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Universität Greifswald

vorgelegt von

Kathrin Jeske

Warendorf, den 06.11.2020

Dekan: Prof. Dr. Gerald Kerth

1. Gutachter: Prof. Dr. Rainer G. Ulrich

2. Gutachter: PD Dr. Sandra Eßbauer

Tag der Promotion: 10.03.2021

II

Summary

More than half of the infectious diseases in humans are caused by zoonotic pathogens or pathogens of animal origin that were transmitted to humans a long time ago. Two important rodent-associated zoonotic pathogens are hantaviruses and human-pathogenic *Leptospira* spp. Both pathogens induce lifelong infection in the rodent hosts that shed the pathogen. Infection with these zoonotic pathogens in humans can cause clinical symptoms. Since some rodents, like the common vole (*Microtus arvalis*) and the bank vole (*Clethrionomys glareolus* syn. *Myodes glareolus*), have cyclic mass reproduction, this can result in years of population outbreaks in an increased number of disease cases in humans. This was found to be the case with the leptospirosis outbreaks in Germany and tularemia outbreaks in Spain, which were traced back to increased common vole density, as well as with the hantavirus disease outbreaks in several European countries, which were associated with bank vole population outbreaks.

The aim of this work was to define the distribution and prevalence of different hantaviruses and leptospires as well as their coinfection in different European rodents, with a focus on voles from the genus *Microtus* and the identification of factors that affect the pathogen prevalence in rodent hosts. Therefore, common voles, bank voles, striped field mice (*Apodemus agrarius*) and other rodents were screened by molecular methods for the presence and prevalence of *Leptospira* spp. and different hantaviruses. Additionally, in selected studies, the presence of anti-hantavirus antibodies was screened by enzyme-linked immunosorbent assay (ELISA) using recombinant hantavirus-nucleocapsid proteins. The prevalence of hantavirus, *Leptospira* spp. and double-infections with both pathogens was analyzed using individual and population-based factors. Small mammals from four different European countries, Spain in the West, Germany and Austria in Central and Lithuania in Northeastern Europe, were included in the studies.

With the molecular screenings, two new hantavirus strains were detected in continental Europe and were named Traemmersee hantavirus (TRAV) and Rusne hantavirus (RUSV) after the trapping locations in Germany and Lithuania, respectively. TRAV was detected in a field vole (*Microtus agrestis*) from the federal state of Brandenburg, Germany, while RUSV was detected in root voles (*Microtus oeconomus*) from Lithuania. Phylogenetic analysis of both hantaviruses indicates their close relation to Tatenale hantavirus and Kielder hantavirus, which were discovered in field voles in Great Britain. A pairwise evolutionary distance (PED) analysis showed that all four hantaviruses belong to the same hantavirus species, for which the putative name "Tatenale orthohantavirus" was proposed. Additionally, a recombinant RUSV antigen

was generated and used successfully in ELISA for the detection of RUSV-specific antibodies and for the analysis of the cross-reactivity of monoclonal and polyclonal antibodies.

In Germany, Tula orthohantavirus (TULV) was foremost detected in common voles in Thuringia and Brandenburg but was also detected in field voles in Brandenburg. Puumala orthohantavirus (PUUV) was detected in Thuringia at the virus distribution border, but sequences differed strongly from known sequences from another neighboring trapping location. While in Austria Dobrava-Belgrade orthohantavirus (DOBV), genotype Kurkino, was detected for the first time in striped field mice, no hantavirus RNA was detected in common voles from Spain. The cause of this absence in the Iberian common vole population might be its long-term isolation from the common vole populations more to the east. The TULV prevalence in Germany in this study was dependent on the season and on the prior growth of the reservoir population. An individual factor that affected the hantavirus prevalence, was the increasing age of the common vole.

Leptospira spp.-DNA was detected in common voles from Spain and Germany, as well as in one striped field mouse from Austria. Except for the two detections of *L. borgpetersenii* in Spain, which were probably the result of spillover infections, only the genomospecies *L. kirschneri* was detected in common voles from Spain and Germany. The high prevalence of *Leptospira* spp., as well as the detection of only one genomospecies, confirm that *L. kirschneri* is the genomospecies for which the common vole is the main reservoir. Important factors for the *Leptospira* spp. prevalence were found to be, in addition to temperature and rainfall, the season and the preceding common vole density. Like the case with hantavirus, the age of the vole was found to be an influencing factor.

In Germany, coinfections of TULV and *Leptospira* spp. were detected. These were associated with high common vole density and increased with the age of the common vole. Furthermore, the incidence of coinfections seems to be impacted more by the *Leptospira* spp. than by the hantavirus prevalence.

As part of this thesis, TULV and PUUV were detected in previously untested regions in Germany, DOBV was detected for the first time in Austria and the distribution range of the putative species "Tatenale orthohantavirus" was extended to continental Europe for the first time with detection in two countries. Screenings in Spain indicate that certain common vole populations can be free from TULV infection. Furthermore, leptospires were detected in rodents from Spain, Germany and Austria. It was verified that certain *Leptospira*

genomospecies are host-specific. Factors that influence the prevalence of infection or coinfection by hantaviruses and leptospires were determined.

The origin and hosts associated with the Tatenale orthohantavirus should be clarified in further studies including the field vole and the root vole as well as other members of the genus *Microtus* in Europe and Asia. The development of a RUSV-antigen-based ELISA will enable future screening in humans and therefore might provide information about the human pathogenicity of this pathogen. For final confirmation of the zoonotic potential, isolation of the virus and development of a focus reduction neutralization test are necessary. The expansion of the striped field mouse to Austria and the detectable carryover of DOBV associated with this implies that further screening studies to more precisely characterize the distribution of DOBV (and other pathogens) are needed. The studies of DOBV spread in Austria as well as PUUV spread in Germany could help to better understand the emergence of zoonotic pathogens in new regions. The here described hantavirus-*Leptospira* spp. and *Neoehrlichia mikurensis-Bartonella* spp. coinfections should be further analyzed to characterize the interactions of the pathogens in the context of a microbiome and their influence on epidemiological aspects of the involved pathogens. The here identified individual and population-based impact factors for the TULV and *Leptospira* spp. prevalence should support the development and optimization of prediction models.

Zusammenfassung

Mehr als die Hälfte der Infektionskrankheiten des Menschen wird durch zoonotische Erreger, die vom Tier auf den Menschen übertragen werden oder vor langer Zeit vom Tier auf den Menschen übergegangen sind, hervorgerufen. Zwei bedeutende Nagetier-assoziierte Zoonoseerreger sind Hantaviren und humanpathogene Leptospiren. Beide Erregergruppen führen im Nagetierwirt zu einer lebenslangen Infektion und Ausscheidung des Erregers und können im Fehlwirt Mensch eine Erkrankung hervorrufen. Da einige Nagetiere, wie die Feldmaus (*Microtus arvalis*) und die Rötelmaus (*Clethrionomys glareolus* syn. *Myodes glareolus*), zyklische Massenvermehrungen zeigen, kann es in den Jahren der Massenvermehrung zur Zunahme der Erkrankungsfälle beim Menschen kommen. So wurden Leptospiroseausbrüche in Deutschland und Tularämieausbrüche in Spanien auf erhöhte Feldmausdichten und Hantavirus-Erkrankungsausbrüche auf Rötelmausmassenvermehrungen in verschiedenen westeuropäischen Ländern zurückgeführt.

Das Ziel dieser Arbeit war die Aufklärung des Vorkommens und der Häufigkeit des Auftretens von verschiedenen Hantaviren und Leptospiren sowie deren Koinfektionen in unterschiedlichen europäischen Nagetieren mit besonderem Fokus auf Wühlmäuse der Gattung *Microtus*, und die Identifikation von Einflussfaktoren auf die Erregerprävalenz in den Nagetierwirten. Hierzu wurden Feldmäuse, Rötelmäuse, Brandmäuse (*Apodemus agrarius*) und andere Nagetiere mittels molekularer Verfahren auf das Vorkommen und die Prävalenz von *Leptospira* spp. und verschiedener Hantaviren untersucht. Für Hantaviren erfolgte zusätzlich bei ausgewählten Studien eine Antikörpertestung mittels Enzyme-linked Immunosorbent Assay (ELISA) unter Verwendung rekombinanter Hantavirus-Nukleokapsidproteine. In die Untersuchungen wurden Kleinsäuger aus vier verschiedenen europäischen Ländern einbezogen: von Spanien im Westen, über Deutschland und Österreich in Mitteleuropa, bis hin zu Litauen im Nordosten.

Im Rahmen der molekularen Untersuchungen wurden in Kontinentaleuropa zwei neue Hantavirusstämme entdeckt, die nach ihren Fangorten in Deutschland und Litauen als Traemmersee-Hantavirus (TRAV) und Rusne-Hantavirus (RUSV) bezeichnet werden. TRAV wurde in einer Erdmaus (*Microtus agrestis*) aus Brandenburg, Deutschland, entdeckt, während RUSV in der nordischen Wühlmaus (*Microtus oeconomus*) in Litauen detektiert wurde. Phylogenetische Analysen der beiden Viren zeigten deren nahe Verwandtschaft mit den in Großbritannien in Erdmäusen gefundenen Tatenale-Hantavirus und Kielder-Hantavirus. Eine Pairwise Evolutionary Distance (PED)-Analyse zeigte, dass diese Viren zu einer

Hantavirusspezies gehören, für die der putative Name „Tatenale-Orthohantavirus" vorgeschlagen wird. Vom RUSV wurde ein rekombinantes Antigen hergestellt und im ELISA erfolgreich für den Nachweis von Virus-spezifischen Antikörpern und die Analyse der Kreuzreaktivität von monoklonalen und polyklonalen Antikörpern eingesetzt.

In Deutschland erfolgte der Nachweis von Tula-Orthohantavirus (TULV) vorwiegend in Feldmäusen (in Thüringen und Brandenburg), aber in Brandenburg auch in Erdmäusen. Das Puumala-Orthohantavirus (PUUV) wurde in Rötelmäusen an der Virusverbreitungsgrenze dieses Virus in Thüringen detektiert, wobei sich die PUUV-Sequenzen stark von bereits vorher nachgewiesenen Sequenzen in Rötelmäusen von einem benachbarten Fangort unterschieden. Während in Österreich erstmals Dobrava-Belgrad-Orthohantavirus (DOBV), Genotyp Kurkino, in Brandmäusen nachgewiesen werden konnte, wurde in Feldmäusen aus Spanien keine Hantavirus-RNA detektiert. Für den fehlenden Nachweis von Hantavirus-RNA in der iberischen Feldmauspopulation könnte deren langanhaltende Isolation von Feldmäusen weiter östlich liegender Populationen die Ursache sein. Die TULV-Prävalenz war in dieser Studie in Deutschland von der Jahreszeit und, zeitlich versetzt, von der vorhergehenden Zunahme der Reservoirpopulation abhängig. Auf der Ebene des Individuums nahm die Hantavirus-Prävalenz mit dem Alter der Feldmaus zu.

Leptospira spp.-DNA wurde in Feldmäusen in Spanien und Deutschland, sowie in einer Brandmaus in Österreich nachgewiesen. Bis auf zwei vermutlich durch Spillover-Infektion bedingte *Leptospira borgpetersenii*-Nachweise in Feldmäusen aus Spanien, wurde in Feldmäusen aus Spanien und Deutschland ausschließlich die Genomospezies *Leptospira kirschneri* detektiert. Die beobachtete hohe Prävalenz von *Leptospira* spp., sowie der ausschließliche Nachweis einer Genomospezies, bestätigten die Feldmaus als maßgeblichen Reservoirwirt für *L. kirschneri*. Als wichtige Einflussfaktoren für die *Leptospira* spp.-Prävalenz wurden neben Temperatur und Niederschlag auch Jahreszeit und vorhergehende Feldmausdichte ermittelt. Auf der individuellen Ebene wurde hier ebenfalls das Alter der Feldmäuse als Einflussfaktor ermittelt.

In Deutschland wurden Koinfektionen von TULV und *Leptospira* spp. nachgewiesen. Diese sind bedingt durch erhöhte Feldmausdichte, sowie zunehmend mit dem Alter der Feldmaus. Darüber hinaus scheint die *Leptospira* spp.-Prävalenz für das Auftreten von Koinfektionen bedeutender als die TULV-Prävalenz.

Im Rahmen dieser Arbeit konnten TULV und PUUV in bisher nicht untersuchten Gebieten in Deutschland, sowie DOBV erstmalig in Österreich nachgewiesen werden und das Verbreitungsgebiet der putativen Art „Tatenale-Orthohantavirus" erstmals auf Festlandeuropa mit Nachweisen in zwei verschiedenen Ländern erweitert werden. Untersuchungen in Spanien deuten an, dass bestimmte Feldmauspopulationen auch frei von TULV-Infektionen sein können. Zudem wurden Leptospiren in Nagetieren aus Spanien, Deutschland und Österreich nachgewiesen und die Wirtsspezifität bestimmter Leptospirengenomospezies bestätigt. Für Hantaviren, Leptospiren und deren Koinfektionen wurden zudem Einflussfaktoren für deren Infektionshäufigkeit bestimmt.

In weiterführenden Untersuchungen unter Einbeziehung von Erdmaus und der nordischen Wühlmaus sowie anderer Arten der Gattung *Microtus* in Europa und Asien sollte die Herkunft und Wirtsassoziation des Tatenale-Orthohantavirus aufgeklärt werden. Die Entwicklung eines RUSV-Antigen-basierten ELISAs wird im Rahmen zukünftiger Untersuchungen beim Menschen zur Aufklärung der Humanpathogenität dieses Erregers beitragen. Für eine finale Bewertung des Zoonosepotenzials dieses Virus ist jedoch die Virusisolierung und darauf basierende Entwicklung eines Fokusreduktionsneutralisationstests erforderlich. Die Einwanderung der Brandmaus in Österreich und die damit scheinbar verbundene Einschleppung des DOBV erfordert weitere gezielte Monitoringuntersuchungen, um die Ausbreitung des DOBV (und weiterer Erreger) genauer zu charakterisieren. Die Untersuchungen zur Ausbreitung von DOBV in Österreich und von PUUV in Deutschland könnten wichtige Hinweise liefern, um das Erstauftreten (Emergence) von Zoonoseerregern in neuen Gebieten besser zu verstehen. Die hier beschriebenen Hantavirus-Leptospiren- und *Neoehrlichia mikurensis-Bartonella* spp.-Koinfektionen sollten zukünftig genauer analysiert werden, um mögliche Interaktionen der Erreger unter Einbeziehung des Mikrobioms und deren Auswirkungen auf die Epidemiologie der beteiligten Erreger zu charakterisieren. Die hier identifizierten individuellen und Populations-basierten Einflussfaktoren für die TULV- und Leptospiren-Prävalenz sollten für die zukünftige Weiterentwicklung von Vorhersagemodellen und deren Optimierung herangezogen werden.

Content

List of Figures

List of Tables

List of abbreviations

Abbreviations

aa	amino acid(s)
bp	base pair(s)
BP	before present
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
HCPS	hantavirus cardiopulmonary syndrome
HFRS	hemorrhagic fever with renal syndrome
kb	kilobase(s)
LGM	Last Glacial Maximum
MLST	multiple-locus sequence typing
NCR	non-coding region
PED	pairwise evolutionary distance
PCR	polymerase chain reaction
RdRP	RNA-dependent RNA polymerase
RNA	ribonucleic acid
RT-PCR	reverse transcription-PCR
ST	sequence type

Virus species abbreviations

ADNV	Andes orthohantavirus
DOBV	Dobrava-Belgrade orthohantavirus
HTNV	Hantaan orthohantavirus
PUUV	Puumala orthohantavirus
RUSV	Rusne hantavirus
SNV	Sin Nombre orthohantavirus
TATV	Tatenale hantavirus
TRAV	Traemmersee hantavirus
TULV	Tula orthohantavirus

1 Introduction

1.1 Zoonoses

1.1.1 Impact of zoonoses

"Zoonoses" are defined as diseases in humans caused through direct or indirect contact with infected animals (World Health Organization 2020). Of the known pathogens, including bacteria, viruses, fungi, prions and parasites, 61% are zoonotic. Furthermore, 75% of pathogens associated with emerging diseases in humans are zoonotic pathogens that can be transmitted from animals to humans (Taylor et al. 2001). The biggest contributor to emerging infectious diseases are bacteria with 54 %, followed by viruses and prions that contribute to 25 % (Jones et al. 2008). Annually, these zoonotic pathogens affect more than one billion people and result in over 500,000 deaths (Webster et al. 2016), as well as massive financial damage (Smith et al. 2019).

An increasing risk factor for human health are zoonotic pathogens of wildlife origin, especially viruses. It is assumed that over 500,000 zoonotic viruses are still undiscovered (Carroll et al. 2018; Jones et al. 2008). Zoonotic viruses, which are associated with characteristics such as aerosol transmission route and low death rate, have been the cause of almost all recent pandemics, including reportedly the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic (Geoghegan et al. 2016; Jones et al. 2008; Ren et al. 2020). Some pathogens originating from wildlife have adapted to humans, such as human immunodeficiency virus 1 and 2 (HIV-1, HIV-2), SARS-CoV-2 or smallpox virus (Ren et al. 2020; Sharp and Hahn 2011; Shchelkunov 2009). More zoonotic RNA than DNA viruses are currently known (Olival et al. 2017). The reason for this is that RNA viruses feature high mutation rates that can make adaption to new hosts easier (Jones et al. 2008). In addition to pathogens that result in pandemics, some cause sporadic outbreaks or local clusters, like those previously seen for hantaviruses and *Leptospira* spp., caused by environmental factors and/or changes in the abundance of the reservoir species (Cann et al. 2013; Desai et al. 2009; Reil et al. 2015).

1.1.2 Reservoirs of zoonotic pathogens

According to the World Health Organization, zoonoses are maintained by a vertebrate, non-human reservoir (World Health Organization 2020). A reservoir host can be defined with the following characteristics (Hallmaier-Wacker et al. 2017; Haydon et al. 2002; Power and Mitchell 2004):

I) The reservoir has an essential part in maintaining the pathogen.

II) This is proven by multiple detections over a period of time and high genetic as well as functional similarity of the detected pathogen.

III) The reservoir host shows no or only limited clinical symptoms due to infection by the zoonotic pathogen.

IV) A transmission route from reservoir to non-reservoir hosts exist, which results in an infection and clinical symptoms of the latter ("spillover infection").

Biodiversity can decrease the prevalence of species-specific pathogens like hantaviruses, as an example through reduction of contacts between the reservoir hosts. This effect is called a "dilution effect". In contrast, the "amplification effect" increases the prevalence of generalist pathogens, such as tick-borne encephalitis virus, when the biodiversity is high, because many different species can act as a reservoir (Johnson et al. 2015).

Anthropogenic factors, such as human urbanization and land use in particular, negatively affect biodiversity (Wilcove et al. 2013). This may also impact species that can act as reservoir hosts for zoonotic pathogens. While some species (like small mammals) disappear and therefore result in loss of biodiversity, other species change habits or habitats or multiply more and are therefore in closer contact to humans and their surroundings. Opposite of the dilution effect, loss of biodiversity can result in an increase in the prevalence of species-specific pathogens in species that are unaffected or profit from anthropogenic influence, e.g. the common vole, resulting in increased risk of infection by zoonotic agents to humans and possibly outbreaks or even pandemics (Hassell et al. 2017; Morand et al. 2019).

1.2 Rodents as reservoirs of zoonotic pathogens

1.2.1 An overview

Rodents represent around 40% of all mammal species and can be found in various habitats worldwide except for Antarctica (Wilson et al. 2016). They function as the most common mammal carrier of zoonotic pathogens with over 60 different known zoonotic pathogens, including hantaviruses and *Leptospira* spp. (Han et al. 2015; Löhmus et al. 2013; Luis et al. 2013; Meerburg et al. 2009; Plourde et al. 2017). Many rodent species carry more than one zoonotic pathogen and coinfections have been detected before (e.g. *Borrelia* spp., hantaviruses, *Leptospira* spp. and *Rickettsia* spp.), increasing the risk of human infection and potentially also even further coinfection (Han et al. 2015; Herbreteau et al. 2012; Kurucz et al. 2018; Schmidt

et al. 2014; Sunil-Chandra et al. 2015; Tadin et al. 2012). The importance of rodents as reservoirs of zoonotic pathogens is highlighted further by their fast life cycle, leading to rapid reproduction of some rodent species, like common voles (*Microtus arvalis*) and bank voles (*Clethrionomys glareolus* syn. *Myodes glareolus*), that allow them to acquire, sustain and transmit various pathogens. Additionally, several rodent species are tolerant of anthropogenic impact on habitats, enabling them to live close to humans and/or livestock, e.g. rats (*Rattus rattus* and *Rattus norvegicus*), the house mouse (*Mus musculus*) and agricultural pests such as the common vole (Plourde et al. 2017). Especially the family Cricetidae and its genus *Microtus* contain many species that can act as hyper-reservoir species (Han et al. 2015). Hotspots for rodent-borne disease outbreaks can be found in North America, Asia and Europe (Han et al. 2015).

1.2.2 Mice and rats

Important rodent species of the family Muridae in Europe include, in addition to the rats and the house mouse, members of the genus *Apodemus*:

I) The yellow-necked mouse *(Apodemus flavicollis)* (Fig. 1A) is widely distributed in European forests, especially in forest edges.

II) The wood mouse (*Apodemus sylvaticus*) (Fig. 1B) also lives in forests, but also in shrubland and grassland.

III) The striped field mouse (*Apodemus agrarius*) (Fig. 1C) lives in forest edges and woodland. This rodent is distributed in two separate populations: in Central and Eastern Europe up to Russia and in China as well as parts of Southeast Asia (Pardiñas et al. 2017).
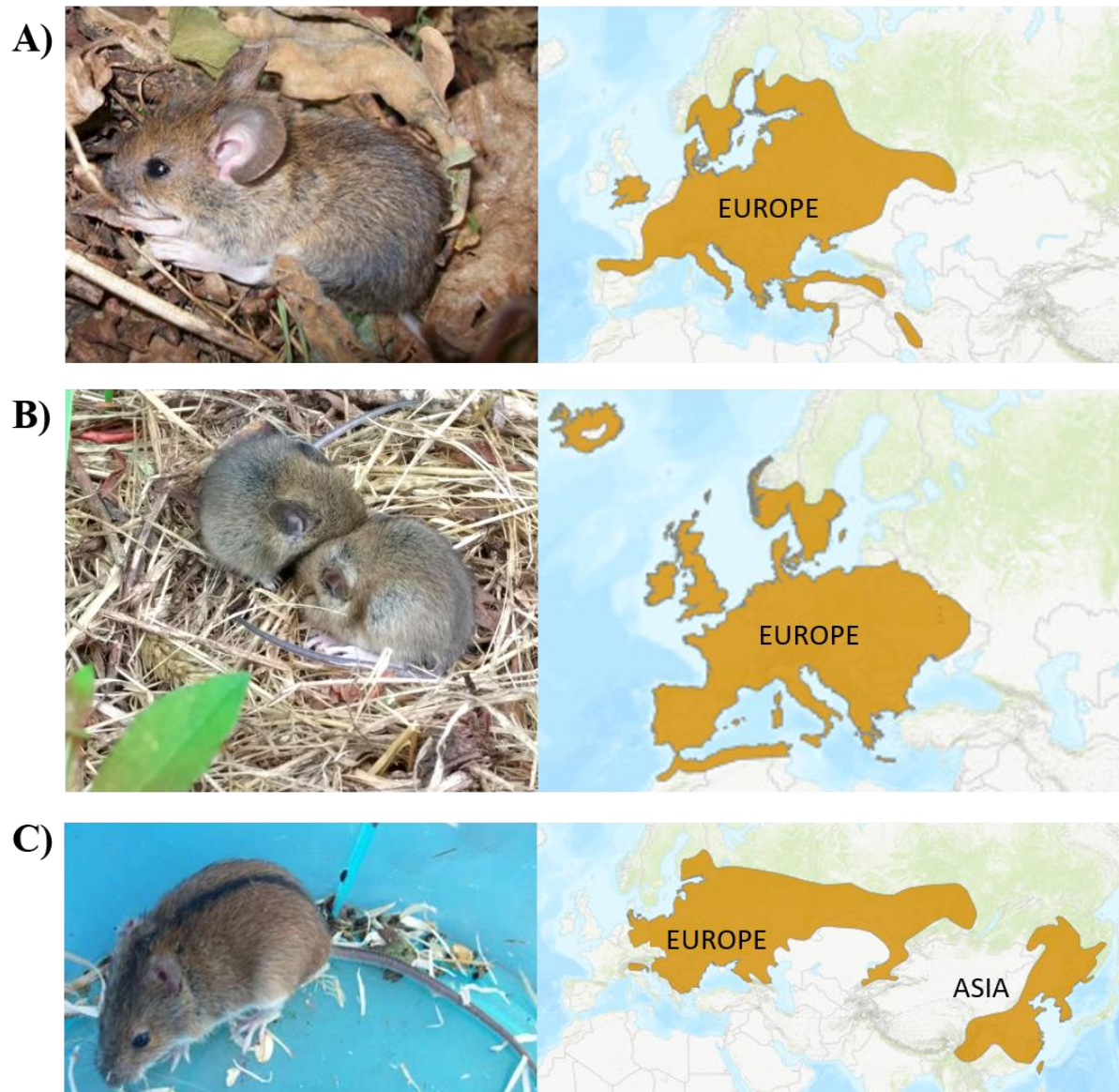
Figure 1:   Photo of rodent (left) and distribution map (right) of yellow-necked mouse (A), wood mouse (B) and striped field mouse (C). Photos by Ulrike M. Rosenfeld and © Manu Cernadas https://www.inaturalist.org/observations/48699601. Distribution maps from IUCN Red List (Amori 2016 ; Kaneko 2016; Schlitter 2016).

1.2.3 Voles

Besides the family Muridae, the family Cricetidae with the subfamily Arvicolinae is of importance, as it carries a large number of zoonotic agents (for examples of pathogens detected in the common vole, see Appendix, Tables S1 - S4). The genus *Microtus* consists of around 60 species, almost 20 species of which can be found in Europe. Some vole species are limited to small geographic areas such as the Lusitanian Pine vole (*Microtus lusitanicus*) (Fig. 2A) in Portugal and Northwestern Spain and the European pine vole (*Microtus subterraneus*) in Central Europe (Fig. 2B).
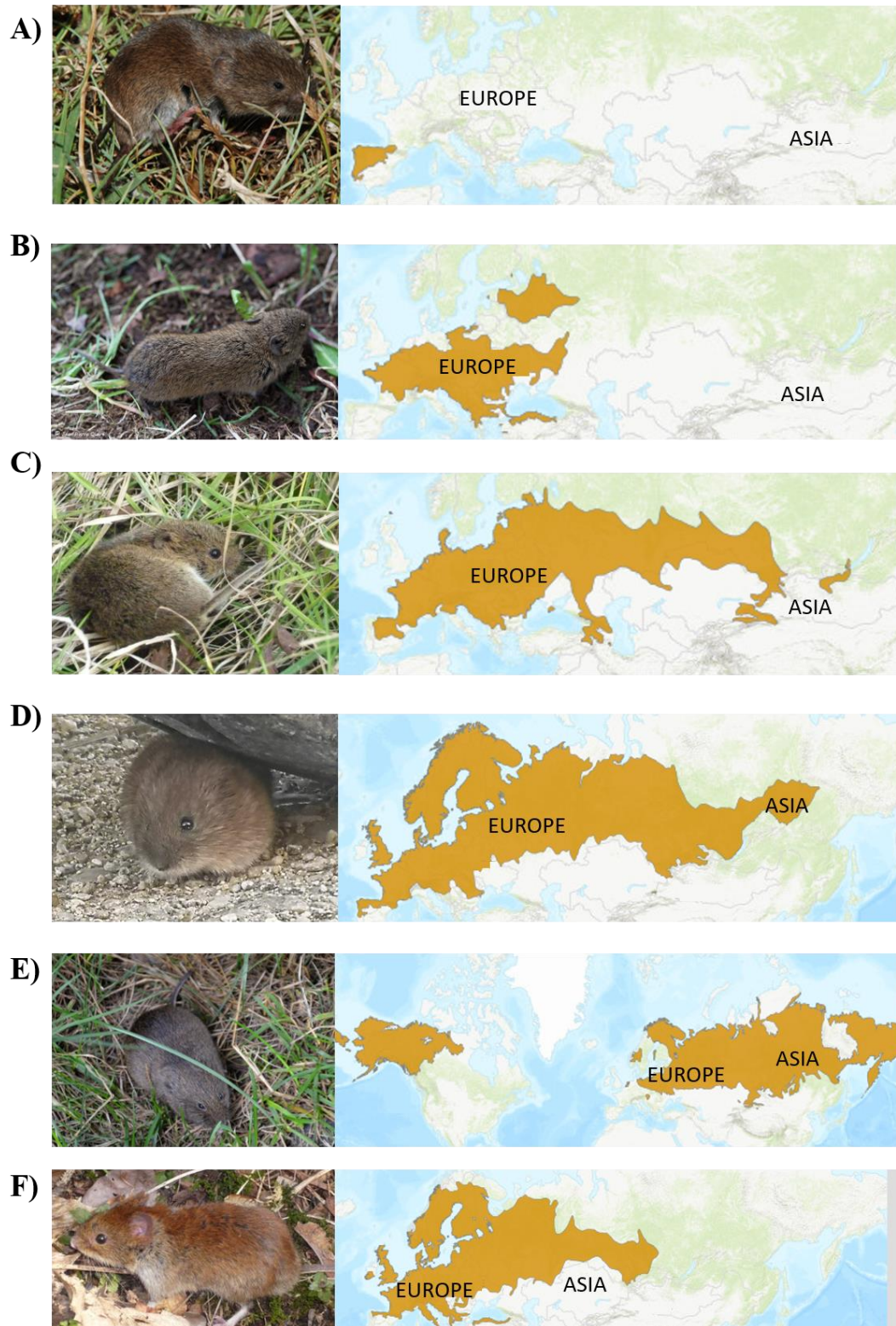
Figure 2: Photo of vole (left) and distribution map (right) of Lusitanian pine vole (A), European pine vole (B), common vole (C), field vole (D), root vole (E) and bank vole (F). Photos by © franciscodocampo https://www.inaturalist.org/observations/45525040, Ulrike M. Rosenfeld, Jean-Pierre Quere, © lukesarti https://www.inaturalist.org/observations/40586763, and © Eva Kundtová Klocová https://www.inaturalist.org/observations/45584234. Distribution maps IUCN Red List (Amori 2016; Aulagnier 2016; Hutterer et al. 2016; Kryštufek 2016; Linzey 2016; Yigit 2016).

Other species like the common vole (Fig. 2C) and the field vole (*Microtus agrestis*) (Fig. 2D) inhabit greater geographical areas such as Europe and parts of Asia. Especially the root vole (also called tundra vole) (*Microtus oeconomus*) can be detected in parts of three continents (Asia, Europe and North America) (Fig. 2E) (Pardiñas et al. 2017). In Europe, the bank vole (Fig. 2F) is found in most parts of Europe up to western parts of Russia, lives in mixed and coniferous forests, shrublands and hedges and prefers vegetation that offers ground cover (Pardiñas et al. 2017).

Common vole population cycles peak every two to five years, with outbreaks of up to more than 2,000 individuals per hectare (Bryja et al. 2005). This does not only lead to massive agricultural damage (Leukers and Jacob 2013), but also to increased risk of pathogen transmission and possible disease outbreaks of rodent-borne pathogens in humans, as reported for the past tularemia outbreaks in Spain (Luque-Larena et al. 2015; Rodriguez-Pastor et al. 2018; Rodriguez-Pastor et al. 2017) and leptospirosis outbreaks in Germany, which were associated with common voles (Dreesman et al. 2016; Hermannsen 1954; Popp 1960).

1.3 *Leptospira* spp. – a bacterial zoonotic pathogen

1.3.1 Classification of leptospires

Leptospires are aerobic spirochetes belonging to order Spirochaetales, family Leptospiraceae, genus *Leptospira*, and they measure 0.1 µm in diameter and 6-20 µm in length (Faine et al. 1999). They are hooked at both ends and have a corkscrew-like appearance (Adler and de la Pena Moctezuma 2010). Leptospires were initially classified as saprophytic or pathogenic species by serological tests (Levett 2001). Currently 35 *Leptospira* spp. are known, which can be divided genetically and pathogenetically into (Thibeaux et al. 2018b):

I)   a saprophytic, non-pathogenic group of environmental bacteria that do not need a host and are rapidly cleared in animal models,

II)   an intermediate group that does not cause disease in the classical animal model but were detected in humans and animals and

III) a pathogenic group (including species detected in rodents like *Leptospira interrogans*, *Leptospira borgpetersenii* and *Leptospira kirschneri*) that can infect every mammal.

The last group is believed to have evolved from the saprophytic species, leading to the establishing of over 400 conserved genes only found in pathogenic leptospires such as the *lipl32* gene encoding the LipL32 subsurface protein. The *lipl32* gene is used for the screening of

pathogenic *Leptospira* spp. by a specific PCR (Lehmann et al. 2014; Mayer-Scholl et al. 2011). The gain of several new genes allowed the different pathogenic *Leptospira* spp. to adapt to a wide variety of hosts (Lehmann et al. 2014). Besides genetic differentiation, the parallel serological differentiation into groups called serovars can be used to classify *Leptospira* spp. (e.g. serovar *grippotyphosa*) and is still used in diagnostics (Adler 2015).

1.3.2 Leptospirosis – The disease

Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* spp. and is of global importance, with 1 million severe cases reported every year and 60,000 deaths annually, not counting the many leptospirosis cases that were misdiagnosed as other diseases (Cosson et al. 2014; Costa et al. 2015; Gouveia et al. 2008). The greatest disease concentration has been shown to be in tropical regions, rural areas and slums (Costa et al. 2015; Reis et al. 2008; Spichler et al. 2008). Disease outbreaks can be triggered by extreme weather events such as flooding, but it can also occur under temperate conditions, for example as smaller disease clusters in Europe (Cann et al. 2013; Dreesman et al. 2016; Morgan et al. 2002; Wynwood et al. 2014). Infection with pathogenic *Leptospira* spp. due to contact with contaminated water or soil is asymptomatic in most cases. In some cases, it can proceed to severe disease forms like Weil's disease, which can result in organ necrosis (fatality rate 10%) or severe pulmonary hemorrhagic syndrome with death by hypoxia (fatality rate 50%) (Adler and de la Pena Moctezuma 2010; Bharti et al. 2003; Ko et al. 2009). The severe symptoms are caused by inflammatory immune responses against leptospires in the infected tissue, thus resulting in different forms of disease, ranging from meningoencephalitis to nephritis, and making initial diagnosis problematic (Ko et al. 2009; Rajapakse et al. 2015). Leptospires are extracellular pathogens, but survival and replication in macrophages has also been observed for *L. interrogans* (Li et al. 2010). Treatment in animals and humans can be achieved by use of penicillin and doxycycline, with the latter also being used as a preventive medicine in case of events that promote infection and leptospirosis outbreaks such as flooding (Marquez et al. 2017). Vaccines are mainly available for veterinary application in livestock and companion animals. Though human vaccines exist, they are accompanied with several problems such as short-term immunity, serious side effects and little to no cross-protection against infection with other serovars.

1.3.3 The reservoirs of *Leptospira* spp.

The different *Leptospira* spp. are adapted to a wide variety of hosts, which has made leptospirosis one of the most common bacterial zoonoses detected in mammals and also in birds, reptiles and fish (Adler and de la Pena Moctezuma 2010; Fornazari 2017; Jobbins and Alexander 2015; Mgode et al. 2014; Xu et al. 2016). *Leptospira* spp. are adapted to a specific reservoir, which is usually chronically infected with leptospires in the proximal kidney tubule. This results in lifelong shedding of the bacteria with the urine of the host and can cause acute disease in non-reservoir hosts such as humans (Adler and de la Pena Moctezuma 2010).

Outside the reservoir, leptospires can survive in water or moist soil for weeks up to months, depending on the conditions, before infecting a new host. Warm and humid conditions like in the tropics can especially prolong survival (Andre-Fontaine et al. 2015; Casanovas-Massana et al. 2018; Khairani-Bejo et al. 2004; Smith and Self 1955). Survival outside the host in the environment is believed to be meditated through the creation of a biofilm with other microbes (Kumar et al. 2015; Ristow et al. 2008; Trueba et al. 2004).

In the reservoir, the infection is believed to be asymptomatic, but internal injuries in kidney tissue have also been observed (Monahan et al. 2009; Torres-Castro et al. 2016). Reservoirs can harbor one or more *Leptospira* species but can be accidental hosts for other species, leading to acute leptospirosis and shedding of fewer leptospires by the accidental host (Andersen-Ranberg et al. 2016; Birnbaum et al. 1998; Miller et al. 2007; Rojas et al. 2010). Important reservoirs for leptospires are rodents. They are present in most parts of the world, live in close contact to humans and livestock and can reach infection rates of 50% or more, depending on season, area and rodent species (Adler and de la Pena Moctezuma 2010; Calderón et al. 2014; Cosson et al. 2014; Diaz 2015; Fischer et al. 2018a; Jobbins and Alexander 2015; Krøjgaard et al. 2009; Vanasco et al. 2003). Beside rodents, other small mammals, such as shrews and bats, can also harbor *Leptospira* spp. (Dietrich et al. 2015; Fischer et al. 2018a; Hurd et al. 2017).

In European countries such as Spain, Austria and Germany, *L. interrogans*, *L. borgpetersenii,* and *L. kirschneri* have previously been detected in rodents (Fischer et al. 2018a; Heuser et al. 2017; Mayer-Scholl et al. 2014; Millan et al. 2018; Obiegala et al. 2016; Schmidt et al. 2014). The detected *Leptospira* genomospecies can vary depending on reservoir. For example, only *L. kirschneri* is detected in common voles and field voles, whereas forest dwelling rodents can harbor *L. kirschneri*, *L. borgpetersenii* and *L. interrogans* and rats can be infected with *L. interrogans* (Fischer et al. 2018a; Heuser et al. 2017; Mayer-Scholl et al. 2014; Obiegala et al.

2016). Further analysis of the sequence type (ST) by using multiple-locus sequence typing (MLST) allows additional insights into the distribution and host specificity of leptospires in small mammal reservoirs (Fischer et al. 2018a).

1.4 Hantaviruses

1.4.1 Classification, structure and genome organization

Hantaviruses are broadly distributed pathogens in Asia, Africa and Europe, as well as North- and South America. Some of the hantavirus species were found to be zoonotic, with 150,000 human cases annually worldwide, and can lead to death, while the potential of other hantaviruses such as Tula orthohantavirus (TULV) and newly detected Tatenale hantavirus (TATV) are still unknown or being discussed (Jonsson et al. 2010; Kruger et al. 2015a; Pounder et al. 2013; Reynes et al. 2015). Hantaviruses are named after the river Hantaan in South Korea, where the striped field mouse was captured from which the first known "hantavirus", named Hantaan virus (under current classification Hantaan orthohantavirus, HTNV), was isolated (Lee et al. 1978). Though the "actual first" hantavirus detection was that of Thottapalayam thottimvirus from a shrew in India in 1964, it was initially classified as an arbovirus (arthropod-borne virus) instead (Carey et al. 1971). More and more hantaviruses were detected over time, not only in rodents (families: Muridae and Cricetidae), but also in insectivores, such as shrews and moles, as well as bats, fish and reptiles (Lee et al. 1978; Plyusnin et al. 1994; Schlegel et al. 2014; Shi et al. 2018).

Hantaviruses are classified by the International Committee on Taxonomy of Viruses (ICTV) as: Realm: *Riboviria*, Kingdom: *Orthornavirinae,* Phylum: *Negarnaviricota*, Subphylum: *Polyploviricotina*, Class: *Ellioviricetes,* Order: *Bunyavirales*, Family: *Hantaviridae*. This includes four subfamilies: *Actantavirinae* (three fish-associated hantavirus species), *Agantavirinae* (one Agnatha (jawless fish)-associated hantavirus species), *Repantavirinae* with one hantavirus species detected in a gecko and *Mammantavirinae* with hantaviruses detected in small mammals. The latter is further divided into genus *Loanvirus* (two bat-associated hantavirus species), genus *Mobatvirus* (two bat- and one mole-associated hantavirus species), genus *Thottimvirus* (two shrew-associated hantavirus species) and genus *Orthohantavirus* (36 species associated with shrews, moles and rodents) (International Committee on Taxonomy of Viruses 2020; Laenen et al. 2019a).

Previously, the hantavirus species definition included detection of the virus in a defined reservoir species, a four-fold difference in a two-way neutralization test and amino acid sequence difference of greater than 7% in nucleocapsid protein and glycoprotein precursor. Recently, the ICTV changed the definition to an only amino acid sequence-based species definition. Currently, a hantavirus needs to have, in a concatenated multiple alignment of nucleocapsid protein (S segment) and glycoprotein (M segment), a pairwise evolutionary distance (PED) value of greater than 0.1 to other known hantavirus species in order to be acknowledged as a new hantavirus species (International Committee on Taxonomy of Viruses 2016; Laenen et al. 2019a). This resulted in several of the old hantavirus species being either abandoned due to missing segment sequences (like Isla Vista virus) or reintroduced under a known species, as happened for Muju and Hokkaido virus, which now belong to the Puumala orthohantavirus (PUUV), and for Saaremaa virus, which was assimilated as a Dobrava-Belgrade orthohantavirus (DOBV) species (International Committee on Taxonomy of Viruses 2016; Maes et al. 2018).

Hantavirus virions are spherical, with a diameter of 80-120 nm (Vaheri et al. 2013b). The negative-orientated and single-stranded RNA genome consists of three segments (see Table 1):

I) a small (S) segment of 1.6-2 kilobases (kb) coding for the nucleocapsid protein and a non-structural protein (only in Arvicolinae-, Neotominae-, and Sigmodontinae-associated hantaviruses),

II) a medium (M) segment of 3.5-3.6 kb encoding for a glycoprotein precursor which is processed to Gc and Gn glycoproteins and

III) a large (L) segment of 6.5 kb coding for an RNA-dependent RNA polymerase (Jääskelainen et al. 2007; Muyangwa et al. 2015; Plyusnin 2002; Schmaljohn 1996).

Table 1:   Genome organization of different hantaviruses.

Reservoir		Hantavirus	Genome (length in nt: 5′-NCR, CDS, 3′-NCR) Protein (size in aa)		
Family	Scientific name		S N/NS	M GPC	L RdRP
Cricetidae	*Clethrionomys glareolus*	Puumala orthohantavirus	42, 1302, 527 433/90	40, 3447, 195 1148	36, 6471, 43 2156
	Microtus arvalis	Tula orthohantavirus	42, 1293, 499 430/90	55, 3426, 213 1141	36, 6462, 43 2153
	Microtus agrestis	Tatenale hantavirus*	n.a., 1302, n.a. 433/90	n.a., 3447, n.a. 1148	n.a., 6465, n.a 2154
	Microtus fortis	Yuanjiang orthohantavirus	42, 1302, n.a. 433/95	n.a., 3438, 197 1145	36, 6471, n.a. 2156
	Microtus maximowiczii	Khabarovsk orthohantavirus	42, 1302, 501 433/95	49, 3435, 222 1144	36, 6465, 78 2154
Muridae	*Apodemus agrarius, A. flavicollis, A. ponticus*	Dobrava-Belgrade orthohantavirus	35, 1290, 34 429/n.c.	40, 3408, 187 1135	37, 6456, 39 2151
Rhinolophidae	*Rhinolophus sinicus*	Longquan loanvirus	54, 1272, 238 423/n.c.	20, 3402, 197 1133	n.a.
Talpidae	*Talpa europaea*	Nova mobatvirus	52, 1287, 486 428/n.c.	20, 3384, 186 1127	33,6474, 56 2157
Soricidae	*Suncus murinus*	Thottapalayam thottimvirus	67, 1308, 155 435/n.c.	39, 3366, 216 1121	62, 6453, 117 2150

n.a. not available; n.c., not coded; *currently not listed as a virus species; CDS, coding sequence; NCR, non-coding region; N, nucleocapsid protein; NS, non-structural protein; GPC, glycoprotein precursor; RdRP, RNA-dependent RNA polymerase.

Puumala orthohantavirus (AJ223376, NC_005223, HE801635), Tula orthohantavirus (NC_005227, NC_005228, AJ005637), Tatenale hantavirus* (MK883757, MK883759, MK883761), Yuanjiang orthohantavirus (FJ170792, KJ857333, KJ857316), Khabarovsk orthohantavirus (NC_034527, NC_034518, KJ857315), Dobrava-Belgrade orthohantavirus (NC_005233, NC_005234, NC_005235), Longquan loanvirus (NC_043126, NC_043127), Nova mobatvirus (NC_034464, NC_034470, NC_034465), Thottapalayam thottimvirus (NC_010704, NC_010708, NC_010707).

1.4.2   Human infection

Inhalation of aerosols of rodent feces containing hantaviruses or biting by infected rodents can lead to human infection (Krüger et al. 2001). Human-to-human transmission is only reported for Andes orthohantavirus (ANDV) (Padula et al. 1998). To date, human disease is only known

for Murinae-associated orthohantaviruses, specifically Seoul orthohantavirus (SEOV), Dobrava-Belgrade orthohantavirus (DOBV) and HTNV, as well as for the Sigmondontinae-associated orthohantavirus ANDV, Neotominae-associated orthohantavirus Sin Nombre orthohantavirus (SNV) and one Arvicolinae-associated hantavirus, i.e. PUUV (Krüger et al. 2001). For other hantaviruses such as TULV and newly detected ones such as TATV, pathogenicity is either under discussion or unknown (Pounder et al. 2013; Reynes et al. 2015).

Disease outcome varies among hantaviruses. ANDV and SNV can cause hantavirus cardiopulmonary syndrome (HCPS), resulting in case fatality rates ranging from 25% to 35%. SEOV, HTNV, DOBV and PUUV can cause hemorrhagic fever with renal syndrome (HFRS) and result in case fatality rates of 0.1% (PUUV), up to 1% (SEOV), 0.5 – 10% (DOBV, depending on the virus, see 1.4.5.3.) or up to 10% (HTNV) (Krüger et al. 2011). The greatest incidences of HFRS are detected in Fennoscandia and amount to 10,000 cases in Europe every year, resulting in the most disease cases in Europe (Heyman et al. 2009; Vaheri et al. 2013a).

Infection with an HFRS-inducing hantavirus can also induce HCPS-like symptoms (Vollmar et al. 2016). A reason for the different clinical symptoms, depending on the hantavirus species, might be found in the binding to different human cellular integrin-receptors by pathogenic and nonpathogenic hantaviruses (Gavrilovskaya et al. 1999; Gavrilovskaya et al. 1998; Geimonen et al. 2002). Other factors might include co-receptors, cell tropism, apoptosis and interaction with the immune system (Ermonval et al. 2016). Increased capillary permeability is the main factor for disease symptoms in the later stages and is caused by a combination of the immune system damaging the barrier function of the capillaries and the virus inducing increased permeability of blood vessels (Jiang et al. 2016; Vaheri et al. 2013b).

ETAR, ribavirin and favipiravir are currently used antihantavirus medications that target the RdRP (Liu et al. 2019). A hantavirus vaccine (Hantavax®) is currently only available in Korea and China. Other vaccines and treatment methods are still under development and/or testing (Brocato and Hooper 2019; Krüger et al. 2011).

1.4.3 Evolution

Hantavirus evolution is associated with the evolution and lineage splitting of their reservoir hosts. As an example, the geographic localization of TULV and PUUV lineages is caused by the geographic distribution of their respective host, common vole and bank vole, respectively, lineages (Castel et al. 2019; Saxenhofer et al. 2019). Hantaviruses which are closely related to

each other are usually harbored by reservoirs which themselves are closely related and coevolve with them, therefore the evolutionary history resembles that of the reservoir (Hjelle and Yates 2001; Shi et al. 2018). Infection of non-reservoir species, so called "spillover events", usually lead either to fast clearance of the virus with no or unspecific symptoms like fever or to the "classical" disease. Alternatively, it is thought that a hantavirus is able to adapt to the new host, replicate and infect a new putative host. This event is called host switching and can happen in species that share the same habitat and therefore increase the chance of infection. It is hypothesized that also closely related species that share important receptors for the virus or have similar immune systems can have an increased chance for this event. It has been discussed whether host switching events occurred during hantavirus evolution (Kang et al. 2009; Ling et al. 2014). Examples are the putative host switch of ancestral DOBV from the yellow-necked mouse to the striped field mouse (Nemirov et al. 2002) and the assumed host switch of an ancestral PUUV from *Clethrionomys* spp. to *Lemmus* spp. and *Microtus* spp. resulting in the evolution of Khabarvosk orthohantavirus and the respective strain Topografov (Vapalahti et al. 1999). Some authors have suggested that host switching dependent on geographical proximity of the reservoir species might be more significant than previously considered for the emergence of new viruses (Rivera et al. 2015). It is believed that the origin of hantaviruses harbored by Murinae and Arvicolinae hosts was in Asia and that spread of the viruses to the other continents was mediated by their respective hosts (Souza et al. 2014). With the finding of hantaviruses in reptiles and fish, it might be assumed that hantaviruses have existed for a longer time period than previously believed (Shi et al. 2018).

1.4.4 The host

The spread of the virus is mediated by the urine, feces and saliva of lifelong-infected reservoirs (Bernshtein et al. 1999; Voutilainen et al. 2015). In addition to wounding and biting, saliva aerosols, which were reported with ANDV transmission, might be an additional transmission route between reservoirs hosts, as saliva was detected with high viral loads (Escutenaire et al. 2002; Padula et al. 2004). Hantavirus infection in the reservoir is believed to be asymptomatic, but negative effects such as decreased survival of PUUV-infected bank voles in winter have been observed (Kallio et al. 2007). In the environment, the virus can persist up to several weeks, depending on the outside conditions such as temperature and UV intensity (Hardestam et al. 2008; Kallio et al. 2006a).

1.4.5 Hantaviruses in Europe

In Europe, at least four hantaviruses exist in rodents: Seoul orthohantavirus in rat, PUUV in bank vole, TULV in common vole and related species, and DOBV in yellow-necked mouse, striped field mouse and Black Sea field mouse (*Apodemus ponticus*) populations (Klempa et al. 2013a; Klempa et al. 2013b). TULV, PUUV and DOBV are discussed in the following sections. A fifth hantavirus circulating in field voles, called TATV, was recently detected in England (Pounder et al. 2013; Thomason et al. 2017). Beside rodent-borne hantaviruses, other hantaviruses such as Brno loanvirus in common noctule (*Nyctalus noctula*) (Straková et al. 2017), Seewis orthohantavirus in common shrew (*Sorex araneus*) (Song J.W. et al. 2007), Asikkala orthohantavirus in Eurasian pygmy shrew (*Sorex minutus*) (Radosa et al. 2013), and Bruges orthohantavirus and Nova mobatvirus in the European mole (*Talpa europaea*) were detected (Gu et al. 2014; Laenen et al. 2018).

1.4.5.1 Tula orthohantavirus

TULV was first detected in Tula, 200 km from Moscow, Russia, in two *Microtus* species, i.e. common voles and East European voles *(Microtus levis* previously known as *M. rossiaemeridionalis*), trapped in 1987 (Plyusnin et al. 1994). Except for other hantaviruses that are detected in a single host, TULV was most often detected in common voles, and it was also detected in other *Microtus* spp. and in *Arvicola* spp. in different countries over time (see Table 2). The geographic clustering of TULV sequences is related with the common vole lineages in Central Europe, as seen for the hybrid zone between Central South and Eastern South TULV lineages at the Bavarian/Czech Republic border (Saxenhofer et al. 2019). Additionally, higher infection rates in common voles compared to other *Microtus* spp. were reported in Central Europe, highlighting that in most cases sympatrically occurring voles might be affected by spillover infections (Schmidt et al. 2016). In regions where common voles are absent, other *Microtus* spp. might act as reservoirs instead (Guo et al. 2019; Polat et al. 2018a; Scharninghausen et al. 2002; Schmidt-Chanasit et al. 2010; Tkachenko et al. 2015). This can result, as in the case of Adler virus in Major's pine vole (*Microtus majori*), in a unique genetic variant of TULV (Tkachenko et al. 2015).

Table 2:   Countries and rodent species in which Tula orthohantavirus was detected by RT-PCR

Country	Rodent species	Reference
Austria	Common vole	(Bowen et al. 1997)
		(Schmidt et al. 2014)
Belgium	Common vole	(Heyman et al. 2002)
China	Altai vole (*Microtus obscurus*)	(Chen et al. 2019; Guo et al. 2019)
Croatia	Common vole	(Scharninghausen et al. 2002; Tadin et al. 2016)
	Field vole	(Scharninghausen et al. 2002)
Czech Republic	Common vole	(Saxenhofer et al. 2019)
France	Common vole	(Schmidt et al. 2016)
Germany	*Arvicola* spp.	(Schlegel et al. 2012; Schmidt et al. 2016)
	Common vole	(Klempa et al. 2003a; Mertens et al. 2011a)
		(Schmidt-Chanasit et al. 2010; Schmidt et al. 2016)
	Field vole	(Schmidt-Chanasit et al. 2010; Schmidt et al. 2016)
Hungary	Bank vole	(Kurucz et al. 2018)
	Common vole	(Kurucz et al. 2018)
Kazakhstan	Altai vole	(Plyusnina et al. 2008a)
Luxembourg	Common vole	(Schmidt et al. 2016)
Netherlands	Common vole	(Maas et al. 2017; Reusken et al. 2008)
Poland	Common vole	(Michalski et al. 2014; Song et al. 2004)
Russia	Common vole	(Plyusnin et al. 1994; Tkachenko et al. 2015)
	Major's pine vole (*Microtus majori*)	(Tkachenko et al. 2015)
	East European voles (*Microtus levis*)	(Plyusnin et al. 1994)
Serbia	Common vole	(Stanojevic et al. 2015)
	European pine vole	(Song et al. 2002)
Slovenia	Common vole	(Korva et al. 2009; Korva et al. 2013)
	European pine vole	(Korva et al. 2009; Korva et al. 2013)
	Field vole	(Korva et al. 2009; Korva et al. 2013)
Slovakia	Common vole	(Sibold et al. 1999a)
Switzerland	Eurasian water vole	(Schlegel et al. 2012)
Turkey	Altai vole	(Polat et al. 2018a)

In contrast to the broad European distribution of common voles and TULV, the number of reported human cases is low. Antibodies against TULV have been detected in tested forest workers in East Germany before (Mertens et al. 2011b). Human disease through TULV infection was observed in only three cases in the Czech Republic, Germany and France, respectively, including the detection of TULV-specific RNA in two of these cases (Klempa et al. 2003a; Reynes et al. 2015; Zelena et al. 2013). Due to the limited number of cases, notably an immunocompromised patient and a child, the zoonotic status of TULV is currently being discussed and TULV is mainly described as nonpathogenic (Krüger et al. 2011).

1.4.5.2 Puumala orthohantavirus

PUUV was first detected by an indirect immunofluorescence test for the detection of antibodies against the causative agent of HFRS (also called nephropathia epidemica). This test used material from bank voles trapped in an HFRS-endemic region, and a positive reaction was found for human serum samples from Puumala, Finland, 1979 (Brummer-Korvenkontio et al. 1980). The hantavirus was later isolated from a bank vole collected in Sweden (Yanagihara et al. 1984). The bank vole is the main reservoir and can be found in most parts of Europe, ranging from northeastern Spain up to Russia (Hutterer et al. 2016). PUUV can be detected in most bank vole-infested continental European countries as well (Olsson et al. 2010). An exception are areas in northeastern Germany. There, mainly Eastern and Carpathian bank vole lineages are present, which do not seem to carry PUUV in northeastern Germany (Drewes et al. 2017a; Drewes et al. 2017b).

Muju virus is carried by the royal vole (*Myodes regulus*) in Korea (Song K.J. et al. 2007), and Hokkaido virus is carried by grey-red backed vole (*Myodes rufocanus*) and Northern red-backed vole (*Myodes rutilus*) in Japan, Buryatia and Siberia (Kariwa et al. 1995; Plyusnina et al. 2008b; Yashina et al. 2015). Both viruses belong, due to the new classification system of the ICTV, to the species Puumala orthohantavirus (see 1.4.1).

Currently, eight distinct genetic PUUV lineages are described that differ in their geographic distribution: 1) the Alpe-Adrian (in Austria, Croatia, Hungary and Slovenia), 2) the Central European (in Belgium, France, Germany, The Netherlands and Slovakia), 3) the Danish (on the island of Fyn in Denmark), 4) the Finnish (in Finland, Russian Karelia and western Siberia), 5) North Scandinavian (in northwestern Finland to northern Sweden), 6) the Latvian (in Latvia, Lithuania and northern Poland), 7) the Russian (in Estonia, Latvia, pre-Ural Russia and southern Poland) and 8) the South Scandinavian (in Norway to Sweden) (Ali et al. 2014; Razzauti et al.

2012; Rosenfeld et al. 2017; Sironen et al. 2001). The strong phylogeographic clustering of PUUV sequences is explained by the coevolution with its host, the bank vole (Castel et al. 2015). PUUV probably originated in Asia in a bank vole ancestor. In an expansion wave, bank voles migrated to Europe, where the strong geographical clustering of both virus and host was shaped by the refugium taken by the host during the last glacial period and subsequent spread of the reservoir population (Castel et al. 2019).

PUUV disease outbreaks in humans are strongly correlated with bank vole population density, as they peak in association with beech masts in the year before, which is linked to increased bank vole population growth (Reil et al. 2015; Reil et al. 2017).

1.4.5.3 Dobrava-Belgrade orthohantavirus

DOBV was initially detected in two areas, i.e. in Dobrava, Slovenia from a yellow-necked mouse and in Belgrade, Serbia from a human patient, and was therefore named after both locations (Avsic-Zupanc et al. 1992; Gligic et al. 1992). The species Dobrava-Belgrade orthohantavirus is currently divided into four genotypes: Kurkino, Saaremaa, Dobrava and Sochi. They might have evolved from an ancestral virus carried by either the striped field mouse (Klempa et al. 2013a) or the yellow-necked mouse (Nemirov et al. 2002).

Genotype Dobrava is named after the first detection of DOBV sequences (Avsic-Zupanc et al. 1992; Gligic et al. 1992). The natural reservoir is the yellow-necked mouse, and the genotype Dobrava has been detected in Albania, Bulgaria, Croatia, Czech Republic, Greece, Italy, Montenegro, Slovakia, Slovenia, Serbia and Turkey (Antoniadis et al. 1996; Avsic-Zupanc et al. 2000; Avsic-Zupanc et al. 1995; Markotic et al. 2002b; Nemirov et al. 2003; Oktem et al. 2014; Papa et al. 2006; Papa and Christova 2011; Plyusnina et al. 2009; Polat et al. 2018b; Rizzoli et al. 2015; Sibold et al. 2001; Taller et al. 1993; Weidmann et al. 2005).

Kurkino, first detected in the Kurkino region of Russia, can be found in the striped field mouse of Denmark, Germany, Hungary, Slovakia, Slovenia and Russia (Kurucz et al. 2018; Nemirov et al. 2004; Plyusnin et al. 1999; Schlegel et al. 2009; Sibold et al. 1999b; Sibold et al. 2001).

Both genotypes, Dobrava and Kurkino, were detected in the same country (Croatia, Hungary, Slovakia and Slovenia) in sympatrically occurring respective hosts without reassortment (Avsic-Zupanc et al. 2000; Jakab et al. 2007a; Jakab et al. 2007b; Klempa et al. 2003b; Plyusnina et al. 2009; Plyusnina et al. 2011; Scharninghausen et al. 1999; Sibold et al. 2001). Spillover infections to other rodents were observed for Dobrava, which was transferred to one

rat species (*R. rattus*), Ural field mouse (*Apodemus uralensis*), edible dormouse (*Glis glis*), wood mouse and house mouse (Oktem et al. 2014; Stanojevic et al. 2015; Weidmann et al. 2005). Spillover infections of Kurkino were detected in yellow-necked mice (Schlegel et al. 2009).

Saaremaa, likewise to Kurkino, was detected in striped field mouse, but up to now exclusively on the islands of Estonia (Saaremaa and Vormsi) (Plyusnin et al. 1997). Saaremaa virus was recognized as an independent virus by the ICTV in the past, but this was abolished and it now belongs to DOBV (Laenen et al. 2019a). Due to unusual clustering of Saaremaa M segment sequences, it is believed that a reassortment event with an older ancestor of Dobrava and Kurkino had taken place (Klempa et al. 2013a).

The fourth genotype, Sochi, was found in the Black Sea field mouse in European Russia (Tkachenko et al. 2005).

Human disease cases with DOBV, though rarer than infections with PUUV, range from severe disease with genotype Dobrava (10-12% fatality rate), moderate disease with Sochi (6% fatality rate) and milder infections with Kurkino (0.3-0.6% death rate) (Dzagurova et al. 2012; Klempa et al. 2013a; Klempa et al. 2008; Kruger et al. 2015b). For Saaremaa virus infection, the danger to humans is currently being discussed, as up to now only antibodies have been detected in human cases and no RNA as definitive proof (Golovljova et al. 2007; Klempa et al. 2013a; Klempa et al. 2013b).

2 Objectives

The knowledge of the occurrence, prevalence and sequence types of hantaviruses and *Leptospira* spp. in Spain, Austria, Lithuania and parts of Germany, i.e. Thuringia, is scarce. In addition, individual and population-related factors driving the prevalence of those two pathogens are largely unknown.

Therefore, the objectives of this study were:

- to study the occurrence of hantaviruses in voles in Lithuania
- to prove the occurrence of single and double infections of hantaviruses and *Leptospira* in voles and other rodents in Spain, Austria and Germany
- to identify the reservoir specificity of the pathogens and study their geographic distribution
- to identify factors that affect pathogen prevalence and coinfections such as individual, population dynamics, and environmental factors.

For this purpose, rodents were trapped in Spain, Germany, Austria and Lithuania and dissected. For hantavirus screening, RNA was extracted from lung samples and screened by hantavirus-specific RT-PCRs. For hantavirus identification, the RT-PCR products were sequenced. In some cases, primer walking and next generation sequencing were performed to generate complete coding sequences. The sequences were then analyzed in phylogenetic trees. In some studies, hantavirus antibodies were additionally detected by ELISA. For pathogenic *Leptospira* spp. screening, kidney DNA was extracted and screened by *lipl32*-PCR. Positive samples were further genotyped by *secY*-PCR and MLST. Biometrical data from trapped rodents, population dynamics and environmental data were used for statistical analysis of hantavirus, *Leptospira* spp. and their coinfection.

3 Publications

3.1 Paper I

Jeske K, Hiltbrunner M, Drewes S, Ryll R, Wenk M, Špakova A, Petraityte-Burneikiene R, Heckel G, Ulrich RG (2019) Field vole-associated Traemmersee hantavirus from Germany represents a novel hantavirus species. Virus genes. 55(6):848-853; doi:10.1007/s11262-019-01706-7

SHORT REPORT

Field vole-associated Traemmersee hantavirus from Germany represents a novel hantavirus species

Kathrin Jeske[1] · Melanie Hiltbrunner[2] · Stephan Drewes[1] · René Ryll[1] · Matthias Wenk[3] · Aliona Špakova[4] · Rasa Petraitytė-Burneikienė[4] · Gerald Heckel[2,5] · Rainer G. Ulrich[1]

Abstract

Vole-associated hantaviruses occur in the Old and New World. Tula orthohantavirus (TULV) is widely distributed throughout the European continent in its reservoir, the common vole (*Microtus arvalis*), but the virus was also frequently detected in field voles (*Microtus agrestis*) and other vole species. TULV and common voles are absent from Great Britain. However, field voles there harbor Tatenale and Kielder hantaviruses. Here we screened 126 field voles and 13 common voles from Brandenburg, Germany, for hantavirus infections. One common vole and four field voles were anti-TULV antibody and/or TULV RNA positive. In one additional, seropositive field vole a novel hantavirus sequence was detected. The partial S and L segment nucleotide sequences were only 61.1% and 75.6% identical to sympatrically occurring TULV sequences, but showed highest similarity of approximately 80% to British Tatenale and Kielder hantaviruses. Subsequent determination of the entire nucleocapsid (N), glycoprotein (GPC), and RNA-dependent RNA polymerase encoding sequences and determination of the pairwise evolutionary distance (PED) value for the concatenated N and GPC amino acid sequences confirmed a novel orthohantavirus species, tentatively named Traemmersee orthohantavirus. The identification of this novel hantavirus in a field vole from eastern Germany underlines the necessity of a large-scale, broad geographical hantavirus screening of voles to understand evolutionary processes of virus–host associations and host switches.

Communicated by Detlev H. Kruger.

✉ Rainer G. Ulrich
rainer.ulrich@fli.de

Kathrin Jeske
kathrin.jeske@fli.de

Melanie Hiltbrunner
melanie.hiltbrunner@iee.unibe.ch

Stephan Drewes
stephan.drewes@fli.de

René Ryll
rene.ryll@fli.de

Matthias Wenk
matthias.wenk@LFB.Brandenburg.de

Aliona Špakova
aliona.spakova@bti.vu.lt

Rasa Petraitytė-Burneikienė
rasa.burneikiene@bti.vu.lt

Gerald Heckel
gerald.heckel@iee.unibe.ch

[1]   Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Südufer 10, 17493 Greifswald-Insel Riems, Germany

[2]   Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

[3]   Landesbetrieb Forst Brandenburg, Fachbereich 4.3 Waldschutz, A.-Möller-Str. 1, 16225 Eberswalde, Germany

[4]   Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio al. 7, 10257 Vilnius, Lithuania

[5]   Swiss Institute of Bioinformatics, Genopode, 1015 Lausanne, Switzerland

Ⓐ Springer

849

Introduction

Hantaviruses (order *Bunyavirales*, family *Hantaviridae*) have been identified in various small mammal reservoirs, including murine and cricetid rodents, shrews, moles, and bats [1]. Rodent-borne hantaviruses are distributed worldwide and can cause hemorrhagic fever with renal syndrome (HFRS) in humans in the Old World [2, 3]. The hantavirus genome is divided into three segments. The large (L) segment encodes the viral RNA-dependent RNA polymerase (RdRP). The medium (M) segment encodes the glycoprotein precursor (GPC) that is co-translationally cleaved into the amino terminal Gn and the carboxy-terminal Gc parts. The small (S) segment encodes the structural nucleocapsid (N) protein [4]. The S segment of Cricetidae-associated hantaviruses encodes in an overlapping open reading frame (ORF) a short putative nonstructural protein (NSs) that functions as an interferon antagonist [5].

There is a high diversity of vole-borne hantaviruses in the Old and New World. Sin Nombre orthohantavirus (SNV) strain Convict Creek, El Moro Canyon orthohantavirus (EMCV), Isla Vista hantavirus (ISLAV), and Prospect Hill orthohantavirus (PHV) have been associated with the California vole (*Microtus californicus*), montane vole (*Microtus montanus*), prairie vole (*Microtus ochrogaster*), and meadow vole *(Microtus pennsylvanicus)* reservoirs in the New World [6–11]. Several vole-borne hantaviruses are distributed in Asia and mainland Europe including Khabarovsk orthohantavirus (KHAV) in reed vole (*Microtus fortis*), Maximowicz's vole (*Microtus maximowiczii*), and tundra vole (*Microtus oeconomus*), KHAV strain Topografov in reed vole, Fusong orthohantavirus (FUSV) in reed vole, and Yakeshi orthohantavirus in Maximowicz's vole [11–13].

Tula orthohantavirus (TULV) has a broad geographical distribution that ranges from France in the west to Russia in the northeast and Turkey in the south [14–20]. In depth analyses of TULV and its rodent hosts in Europe have recently led to the identification of independently evolving TULV clades associated with the evolutionary lineages Central (CEN) or Eastern (EST) in the common vole (*Microtus arvalis*) [19]. Within each of these host lineages, two deeply divergent TULV clades occur named after their nonoverlapping geographical ranges TULV Central North (CEN.N) and Central South (CEN.S) or Eastern North (EST.N) and Eastern South (EST.S) [18, 20]. TULV has also been detected in other vole species, i.e., field vole (*Microtus agrestis*), narrow-headed vole (*Microtus gregalis*), East-European vole (*Microtus levis* formerly *Microtus rossiaemeridionalis*), Altai vole (*Microtus obscurus*), European pine vole (*Microtus subterraneus*), Major's pine vole (*Microtus majori*), and water vole (*Arvicola* spp.)

[12–15, 17, 21, 22]. However, the long-term evolution of TULV appears to be associated with the common vole as suggested by the isolation-by-distance (IBD) relationship between virus strains across Europe that is driven by the genetic diversity of TULV found in this reservoir species [20].

Currently, little is known about the role of the field vole as hantavirus reservoir. TULV RNA detection in field voles is discussed as a result of spillover infection from sympatric common vole reservoir [17, 18]. Field voles from the British Isles were found to be a reservoir for Tatenale hantavirus, a virus only distantly related to TULV [23]. A related sequence was detected more recently in field voles from Kielder Forest in England, 230 km from the area where Tatenale hantavirus was initially detected [24]. To test for the potential role of field voles as reservoir of TULV and its host specificity in sympatry, we collected field voles and common voles in Brandenburg, eastern Germany, including regions where TULV was previously almost exclusively detected in field voles [17].

A total of 126 field voles and 13 common voles were collected in 2006, 2007, 2008, and 2018 at four trapping sites in Brandenburg (Fig. 1a) and dissected according to the standard protocol [25]. Enzyme-linked immunosorbent assay (ELISA) screening of chest cavity fluids (CCF) was performed in parallel with *Saccharomyces cerevisiae*-produced N proteins of two TULV strains, strain Thuringia [26], and strain Moravia [19, 22, 27]. Here, four of 126 field voles and one of 13 common voles from two trapping sites were detected in both ELISAs as seropositive (Table 1).

Screening of lung tissue-derived RNA by conventional reverse transcription-polymerase chain reaction (RT-PCR) assays targeting the S segment [22], M segment [28], and L segment [29] resulted in specific amplification products in three of 126 field voles and one of 13 common voles (Table 1). The common vole and one field vole were anti-TULV antibody and TULV RNA positive. Two field voles were exclusively anti-TULV positive, but RT-PCR negative, whereas an additional field vole was only TULV RNA positive (Table S1).

For phylogenetic analysis, additional 12 S, 31 M, and 32 L segment TULV sequences were generated for TULV strains that were identified in a previous study (Ref. [18]; Table S2). Direct sequencing of the S and M segment RT-PCR products of three voles of the current study resulted in the identification of sequences from the TULV-CEN.N clade in two field voles collected at Stadtsee and in one common vole from Traemmersee (Fig. S1A and B). The novel partial L segment sequences also clustered within the TULV-CEN.N clade (data not shown). Cytochrome *b* (*cytb*) analysis revealed that the TULV RNA-positive common vole belongs to the evolutionary lineage Central in this species
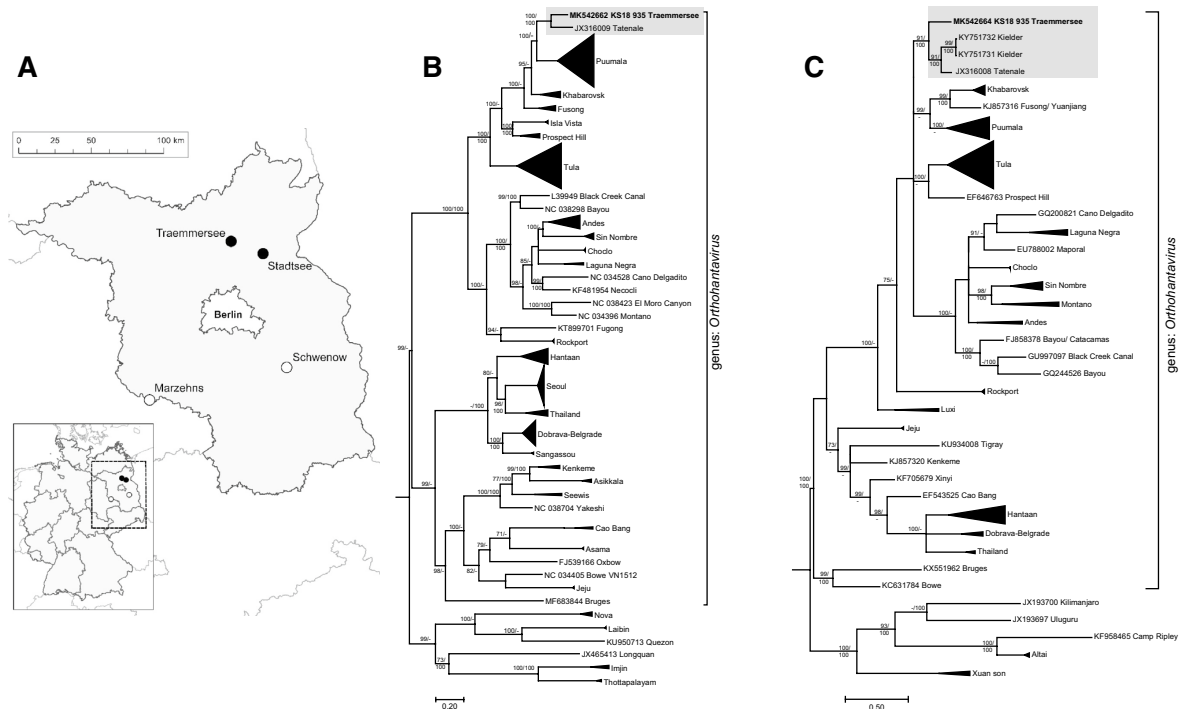
Fig. 1 Map of vole trapping sites and phylogenetic trees of partial S and L segment sequences of hantaviruses including the new Traemmersee orthohantavirus and the British Tatenale and Kielder hantaviruses. **a** Map of the trapping sites of field voles (*Microtus agrestis*) and common voles (*Microtus arvalis*) in Brandenburg, eastern Germany. Trapping sites of hantavirus-RNA-positive field voles and common voles are indicated by filled circles. **b** Consensus phylogenetic tree of the partial S segment sequences (alignment length 393 nucleotides (nt), positions 622–1003, counting according to Tula orthohantavirus (TULV) S segment, accession number NC_005227). **c** Consensus phylogenetic tree of partial L segment sequences (alignment length 333 nt, positions 2983–3309, counting according to TULV L segment, accession number NC_005226). Phylogenetic trees for partial S and L segment sequences were constructed because for Tatenale and Kielder hantaviruses only partial sequences are available. The consensus trees are based on Bayesian analyses with 15,000,000 generations and a burn-in phase of 25%, and maximum-likelihood analyses, with 1000 bootstraps and 50% cutoff using the general time reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter for both algorithms. Posterior probabilities in percent from Bayesian analyses are given before the slash and bootstrap values are given after the slash for major nodes when they exceeded 70. The tree reconstructions were done via CIPRES [37]. Alignments were constructed under Bioedit (V7.2.3.) [38] using the Clustal W Multiple Alignment algorithm implemented in the program. Names in bold indicate newly generated sequences (MK542662 and MK542664) and field vole viruses clustering with Traemmersee virus are highlighted by a gray box. Triangles indicate compressed branches. Additional accession numbers are listed in Table S6

which is consistent with large-scale phylogeographic patterns (Refs. [19, 30]; Table S1, Fig. S2A).

Interestingly, S and L segment sequences from a single seropositive field vole from Traemmersee were highly divergent to TULV, but similar to the British field vole-associated Tatenale and Kielder hantaviruses (Fig. 1b, c, Table S3). In addition, RT-PCR amplified partial GPC-encoding sequence was also highly divergent from TULV M segment sequences (identity of 75–80%; Fig. S1B); M segment sequences from Tatenale and Kielder hantaviruses are not available so far.

The complete coding sequences of S, M, and L segments were generated by a primer-walking approach (for primers used see Table S4). The encoded N protein, GPC, and RdRP are 433, 1148, and 2154 amino acids in length, respectively.

A moderate similarity to TULV and a higher similarity to the sequences of other vole-borne hantaviruses were identified by pairwise comparison of the nucleotide and amino acid sequences as well as in the phylogenetic trees (Table S5 and Fig. S3A–F). A 270 nucleotide-long NSs ORF overlapping the N ORF was identified; the amino terminal region of the putative NSs protein is similar to that of the majority of vole-borne hantaviruses, but differs to the amino-terminally extended NSs proteins of KHAV and FUSV (data not shown).

The pairwise evolutionary distance (PED) values of the concatenated N protein and GPC of Traemmersee virus and KHAV, FUSV, Puumala orthohantavirus (PUUV), PHV, and TULV vary between 0.14 and 0.66 (Table S5). These values

Table 1 Results of the serological and RT-PCR investigations of field voles (*Microtus agrestis*) and common voles (*Microtus arvalis*) from four trapping sites in eastern Germany

Trapping site (see Fig. 1a)	Trapping year	Species	Total number of voles trapped	Results (number positive/total number tested)			Virus (lineage)
				IgG ELISA TULV EST.S (Moravia) N protein	IgG ELISA TULV-CEN.N (Thuringia[a]) N protein	S segment RT-PCR	
Marzehns	2006	*M. agrestis*	9	0/9	0/9	0/9	
	2007	*M. agrestis*	33	0/33	0/33	0/33	
		M. arvalis	7	0/7	0/7	0/7	
Schwenow	2006	*M. agrestis*	5	0/5	0/5	0/5	
	2008	*M. agrestis*	3	0/3	0/3	0/3	
		M. arvalis	1	0/1	0/1	0/1	
Stadtsee	2006	*M. agrestis*	12	0/12	0/12	0/12	
	2007	*M. agrestis*	14	0/14	0/14	0/14	
	2008	*M. agrestis*	39	3/39	3/39	2/39	TULV (CEN.N, Central North)
Traemmersee	2008	*M. agrestis*	8	1/8	1/8	1/8	Traemmersee virus
		M. arvalis	5	1/ 5	1/5	1/5	TULV (CEN.N, Central North)
	2018	*M. agrestis*	3	0/3	0/3	0/3	
Total		*M. agrestis*	126	4/126	4/126	3/126	
		M. arvalis	13	1/13	1/13	1/13	
		all *Microtus*	139	5/139	5/139	4/139	

[a]Amino-terminally his-tagged nucleocapsid (N) protein of Tula orthohantavirus (TULV) strain from Thuringia was produced in yeast *Saccharomyces cerevisiae* and purified by nickel-chelate affinity chromatography under denaturing conditions as described previously [27]

were higher than the cutoff value (0.1) that was defined by the International Committee on Taxonomy of Viruses (ICTV) for a hantavirus species using *D*iv*E*rsity p*A*rtitioning by hie*R*archical *C*lustering (DEmARC) [31]. According to the criteria of the ICTV [31], this suggests a novel virus species that was tentatively named according to the trapping site of the field vole "Traemmersee orthohantavirus" (TRAV). The definition of a novel virus species is also supported by the lack of evidence for recombination of the entire S, M, and L segment nucleotide sequences of TRAV and corresponding reference sequences in SimPlot and RDP4 analyses (Refs. [32, 33]; data not shown). Comparison of the partial N protein sequence of Tatenale hantavirus (131 aa) and TRAV revealed a PED value of 0.0395, which may indicate that Tatenale hantavirus and TRAV represent a single orthohantavirus species.

The paucity of available data on British Tatenale and Kielder hantaviruses and TRAV from other locations prevents at present conclusive analyses of their evolutionary history. However, field voles colonized the British Isles only after the last glaciation less than 15,000 years ago [34–36] which suggests a continental origin of the ancestor of these British hantaviruses. At present, we can only speculate that the evolutionary history of these hantaviruses might be associated with the history of the Western *cytb* lineage in the field vole because the British Isles were colonized by this

lineage [36] and the field vole population at the sampling location of TRAV harbors both the Central and Western lineages.

In conclusion, we identified a novel orthohantavirus species in Germany which is most similar to British hantaviruses detected in the same vole host species. More hantavirus data based on large-scale geographical screening will be necessary to understand the evolutionary history of this system better. However, detailed analyses of TULV clades and evolutionary lineages in the common vole have recently demonstrated that speciation processes in hantaviruses can be triggered by evolutionary divergence in their hosts and may even outrun host evolution [19]. It seems thus appropriate to explicitly consider not only the presumed reservoir host but also related species as potential hosts for a better understanding of the role of host association and host switches in the evolution of hantaviruses.

Author contributions RGU and GH designed the study. MW collected all voles. KJ performed all molecular and serological investigations including sequence determination and analyses and contributed to the

generation of the N antigen of TULV strain Thuringia. MH performed RDP4 and SimPlot analyses. AS and RP contributed to the generation of both TULV N antigens. SD, RR, and GH supervised the sequence analyses. RR performed the PED determination. KJ, GH, and RGU wrote the manuscript draft. All authors contributed to the final version of the manuscript and approved it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The collection of voles was performed by the local forestry institutions during the vole monitoring as part of their pest control measures.

References

1. Forbes KM, Sironen T, Plyusnin A (2018) Hantavirus maintenance and transmission in reservoir host populations. Curr Opin Virol 28:1–6. https://doi.org/10.1016/j.coviro.2017.09.003
2. Jiang H, Zheng X, Wang L, Du H, Wang P, Bai X (2017) Hantavirus infection: a global zoonotic challenge. Virol Sinica 32(1):32–43. https://doi.org/10.1007/s12250-016-3899-x
3. Johnson KM (2001) Hantaviruses: history and overview. Curr Top Microbiol Immunol 256:1–14
4. Elliott RM (1990) Molecular biology of the Bunyaviridae. J Gen Virol 71(3):501–522
5. Vera-Otarola J, Solis L, Soto-Rifo R, Ricci EP, Pino K, Tischler ND, Ohlmann T, Darlix JL, Lopez-Lastra M (2012) The *Andes hantavirus* NSs protein is expressed from the viral small mRNA by a leaky scanning mechanism. J Virol 86(4):2176–2187. https://doi.org/10.1128/jvi.06223-11
6. Song W, Torrez-Martinez N, Irwin W, Harrison FJ, Davis R, Ascher M, Jay M, Hjelle B (1995) Isla Vista virus: a genetically novel hantavirus of the California vole *Microtus californicus*. J Gen Virol 76(Pt 12):3195–3199. https://doi.RDP4/0022-1317-76-12-3195
7. Turell MJ, Korch GW, Rossi CA, Sesline D, Enge BA, Dondero DV, Jay M, Ludwig GV, Li D, Schmaljohn CS et al (1995) Short report: prevalence of hantavirus infection in rodents associated with two fatal human infections in California. Am J Trop Med Hyg 52(2):180–182. https://doi.org/10.4269/ajtmh.1995.52.180
8. Rowe JE, St Jeor SC, Riolo J, Otteson EW, Monroe MC, Henderson WW, Ksiazek TG, Rollin PE, Nichol ST (1995) Coexistence of several novel hantaviruses in rodents indigenous to North America. Virology 213(1):122–130. https://doi.org/10.1006/viro.1995.1552
9. Hjelle B, Jenison SA, Goade DE, Green WB, Feddersen RM, Scott AA (1995) Hantaviruses: clinical, microbiologic, and epidemiologic aspects. Crit Rev Clin Lab Sci 32(5–6):469–508. https://doi.org/10.3109/10408369509082592
10. Lee PW, Amyx HL, Yanagihara R, Gajdusek DC, Goldgaber D, Gibbs CJ Jr (1985) Partial characterization of Prospect Hill virus

11. isolated from meadow voles in the United States. J Infect Dis 152(4):826–829
11. Maes P, Alkhovsky SV, Bào Y, Beer M, Birkhead M, Briese T, Buchmeier MJ, Calisher CH, Charrel RN, Choi IR (2018) Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. Arch Virol 163(8):2295–2310
12. Milholland MT, Castro-Arellano I, Suzan G, Garcia-Pena GE, Lee TE Jr, Rohde RE, Alonso Aguirre A, Mills JN (2018) Global diversity and distribution of hantaviruses and their hosts. EcoHealth 15(1):163–208. https://doi.org/10.1007/s10393-017-1305-2
13. Zou Y, Wang JB, Gaowa HS, Yao LS, Hu GW, Li MH, Chen HX, Plyusnin A, Shao R, Zhang YZ (2008) Isolation and genetic characterization of hantaviruses carried by Microtus voles in China. J Med Virol 80(4):680–688. https://doi.org/10.1002/jmv.21119
14. Polat C, Ergunay K, Irmak S, Erdin M, Brinkmann A, Cetintas O, Cogal M, Sozen M, Matur F, Nitsche A, Oktem IMA (2018) A novel genetic lineage of *Tula orthohantavirus* in Altai voles (*Microtus obscurus*) from Turkey. Infect Genet Evol 67:150–158. https://doi.org/10.1016/j.meegid.2018.11.015
15. Plyusnin A, Vapalahti O, Lankinen H, Lehvaslaiho H, Apekina N, Myasnikov Y, Kallio-Kokko H, Henttonen H, Lundkvist A, Brummer-Korvenkontio M et al (1994) Tula virus: a newly detected hantavirus carried by European common voles. J Virol 68(12):7833–7839
16. Artois M, Cochez C, Van Mele R, Heyman P (2007) Genetic evidence of Puumala and Tula Hantaviruses in rodents in the Jura region, France—preliminary results. Eur Surveill 12(6):E070628
17. Schmidt-Chanasit J, Essbauer S, Petraityte R, Yoshimatsu K, Tackmann K, Conraths FJ, Sasnauskas K, Arikawa J, Thomas A, Pfeffer M, Scharninghausen JJ, Splettstoesser W, Wenk M, Heckel G, Ulrich RG (2010) Extensive host sharing of central European Tula virus. J Virol 84(1):459–474. https://doi.org/10.1128/jvi.01226-09
18. Schmidt S, Saxenhofer M, Drewes S, Schlegel M, Wanka KM, Frank R, Klimpel S, von Blanckenhagen F, Maaz D, Herden C, Freise J, Wolf R, Stubbe M, Borkenhagen P, Ansorge H, Eccard JA, Lang J, Jourdain E, Jacob J, Marianneau P, Heckel G, Ulrich RG (2016) High genetic structuring of *Tula hantavirus*. Arch Virol 161(5):1135–1149. https://doi.org/10.1007/s00705-016-2762-6
19. Saxenhofer M, Schmidt S, Ulrich RG, Heckel G (2019) Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus. PLoS Biol 17(2):e3000142. https://doi.org/10.1371/journal.pbio.3000142
20. Saxenhofer M, Weber de Melo V, Ulrich RG, Heckel G (2017) Revised time scales of RNA virus evolution based on spatial information. Proc Biol Sci. https://doi.org/10.1098/rspb.2017.0857
21. Tkachenko EA, Witkowski PT, Radosa L, Dzagurova TK, Okulova NM, Yunicheva YV, Vasilenko L, Morozov VG, Malkin GA, Kruger DH, Klempa B (2015) Adler hantavirus, a new genetic variant of Tula virus identified in Major's pine voles (*Microtus majori*) sampled in southern European Russia. Infect Genet Evol 29:156–163. https://doi.org/10.1016/j.meegid.2014.11.018
22. Schlegel M, Kindler E, Essbauer SS, Wolf R, Thiel J, Groschup MH, Heckel G, Oehme RM, Ulrich RG (2012) Tula virus infections in the Eurasian water vole in Central Europe. Vector Borne Zoonotic Dis 12(6):503–513. https://doi.org/10.1089/vbz.2011.0784
23. Pounder KC, Begon M, Sironen T, Henttonen H, Watts PC, Voutilainen L, Vapalahti O, Klempa B, Fooks AR, McElhinney LM (2013) Novel hantavirus in wildlife, United Kingdom. Emerg Infect Dis 19(4):673–675. https://doi.org/10.3201/eid1904.121057
24. Thomason AG, Begon M, Bradley JE, Paterson S, Jackson JA (2017) Endemic Hantavirus in field voles, Northern England. Emerg Infect Dis 23(6):1033–1035. https://doi.org/10.3201/eid2306.161607

25. Fischer S, Mayer-Scholl A, Imholt C, Spierling NG, Heuser E, Schmidt S, Reil D, Rosenfeld UM, Jacob J, Nockler K, Ulrich RG (2018) Leptospira genomospecies and sequence type prevalence in small mammal populations in Germany. Vector Borne Zoonotic Dis 25:256. https://doi.org/10.1089/vbz.2017.2140

26. Jeske K, Imholt C, Jacob J, Spakova P, Petraitytė-Burneikienė R, Ulrich RG (unpublished data)

27. Razanskiene A, Schmidt J, Geldmacher A, Ritzi A, Niedrig M, Lundkvist A, Kruger DH, Meisel H, Sasnauskas K, Ulrich R (2004) High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast Saccharomyces cerevisiae. J Biotechnol 111(3):319–333. https://doi.org/10.1016/j.jbiotec.2004.04.010

28. Mertens M, Kindler E, Emmerich P, Esser J, Wagner-Wiening C, Wolfel R, Petraityte-Burneikiene R, Schmidt-Chanasit J, Zvirbliene A, Groschup MH, Dobler G, Pfeffer M, Heckel G, Ulrich RG, Essbauer SS (2011) Phylogenetic analysis of Puumala virus subtype Bavaria, characterization and diagnostic use of its recombinant nucleocapsid protein. Virus Genes 43(2):177–191. https://doi.org/10.1007/s11262-011-0620-x

29. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Denys C, Koivogui L, ter Meulen J, Kruger DH (2006) Hantavirus in African wood mouse, Guinea. Emerg Infect Dis 12(5):838–840. https://doi.org/10.3201/eid1205.051487

30. Lischer HE, Excoffier L, Heckel G (2014) Ignoring heterozygous sites biases phylogenomic estimates of divergence times: implications for the evolutionary history of Microtus voles. Mol Biol Evol 31(4):817–831. https://doi.org/10.1093/molbev/mst271

31. ICTV (2018). https://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/animal-dsrna-and-ssrna--viruses/8066. Accessed 13 July 2019

32. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B (2015) RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol 1(1):vev003. https://doi.org/10.1093/ve/vev003

33. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC (1999) Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 73(1):152–160

34. Herman JS, McDevitt AD, Kawalko A, Jaarola M, Wojcik JM, Searle JB (2014) Land-bridge calibration of molecular clocks and the post-glacial colonization of Scandinavia by the Eurasian field vole *Microtus agrestis*. PLoS ONE 9(8):e103949. https://doi.org/10.1371/journal.pone.0103949

35. Herman JS, Searle JB (2011) Post-glacial partitioning of mitochondrial genetic variation in the field vole. Proc Biol Sci 278(1724):3601–3607. https://doi.org/10.1098/rspb.2011.0321

36. Kotlik P, Markova S, Konczal M, Babik W, Searle JB (2018) Genomics of end-Pleistocene population replacement in a small mammal. Proc Biol Sci. https://doi.org/10.1098/rspb.2017.2624

37. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: 2010 gateway computing environments workshop (GCE), IEEE, pp 1–8

38. Hall T (2011) BioEdit: an important software for molecular biology. GERF Bull Biosci 2(1):60–61

Springer

Table S1: RT-PCR and ELISA results for field voles (*Microtus agrestis*) and common voles (*Microtus arvalis*) that are positive in at least one assay.

ID	Trapping site	Date of capture	Species	Sex	Mass (g)	RT-PCR	Sequence	Accession numbers	IgG-ELISA (TULV EST.S N protein)	IgG-ELISA (TULV CEN.N N protein)
KS18/935	Traemmersee	13.11.2008	*M. agrestis*	male	33.9	pos	Traemmersee virus	MK542662 (S) MK542663 (M) MK542664 (L) MK535087 (*cytb*)	pos	pos
KS18/936	Traemmersee	13.11.2008	*M. arvalis*	male	14.9	pos	TULV-CEN.N	MK535084 (S) MK535069 (M) MK535034 (L) MK535088 (*cytb*)	pos	pos
KS18/940	Stadtsee	01.10.2008	*M. agrestis*	female	28.3	pos	TULV-CEN.N	MK535085 (S) MK535070 (M) MK535035 (L) MK535089 (*cytb*)	neg	neg
KS18/958	Stadtsee	15.07.2008	*M. agrestis*	male	48.8	neg	-	MK535090 (*cytb*)	pos	pos
KS18/963	Stadtsee	15.07.2008	*M. agrestis*	male	40.1	pos	TULV-CEN.N	MK535086 (S) MK535071 (M) MK535036 (L) MK535091 (*cytb*)	pos	pos
KS18/966	Stadtsee	15.07.2008	*M. agrestis*	male	42.1	neg	-	MK535092 (*cytb*)	pos	pos

pos, positive; neg, negative; S, S segment; M, M segment; L, L segment; *cytb*, cytochrome *b*; TULV, Tula orthohantavirus; EST.S, Eastern South; CEN.N, Central North; N, nucleocapsid.

Table S2: Accession numbers for all new Tula orthohantavirus sequences.

Sequence ID	Vole ID	Country/Site	Host	Isolation source	Note	Accession numbers	Identical to
Tula_virus_KS08_260_S	KS08/260	Germany: Warburg	*Microtus arvalis*	lung	S segment	MK535072	
Tula_virus_KS08_277_S	KS08/277	Germany: Warburg	*Microtus arvalis*	lung	S segment	MK535073	
Tula_virus_KS08_350_S	KS08/350	Germany: Gotha	*Microtus arvalis*	lung	S segment	MK535074	
Tula_virus_KS08_639_S	KS08/639	Germany: Westergellersen	*Microtus arvalis*	lung	S segment	MK535075	
Tula_virus_KS08_1045_S	KS08/1045	Germany: Westergellersen	*Microtus arvalis*	lung	S segment	MK535076	
Tula_virus_KS09_1477_S	KS09/1477	Germany: Groß Schoenebeck	*Microtus arvalis*	lung	S segment	MK535077	
Tula_virus_KS09_1669_S	KS09/1669	Germany: Huehnerwasser	*Microtus arvalis*	lung	S segment	MK535078	KU139554 (KS09/1648)
Tula_virus_KS09_2375_S	KS09/2375	Germany: Jeeser	*Microtus arvalis*	lung	S segment	MK535079	
Tula_virus_KS10_29_S	KS10/29	Germany: Jeeser	*Microtus arvalis*	lung	S segment	MK535080	
Tula_virus_KS10_905_S	KS10/905	Germany: Treben	*Microtus arvalis*	lung	S segment	MK535081	KU139578 (KS10/932); MK53082 (KS10/908)
Tula_virus_KS10_908_S	KS10/908	Germany: Treben	*Microtus arvalis*	lung	S segment	MK535082	KU139578 (KS10/932); MK53081 (KS10/905)
Tula_virus_KS10_1661_S	KS10/1661	Germany: Huehnerwasser	*Microtus arvalis*	lung	S segment	MK535083	
Tula_virus_D34_M	D34	Germany: Berlin	*Microtus arvalis*	lung	M segment	MK535038	
Tulv_virus_KS07_862_M	KS07/862	Germany: Tremt	*Microtus arvalis*	lung	M segment	MK535039	

Table S2 (continued)

Sequence ID	Vole ID	Country/Site	Host	Isolation source	Note	Accession Numbers	Identical to
Tula_virus_KS08_260_M	KS08/260	Germany: Warburg	*Microtus arvalis*	lung	M segment	MK535040	MK535041 (KS08/277)
Tula_virus_KS08_277_M	KS08/277	Germany: Warburg	*Microtus arvalis*	lung	M segment	MK535041	MK535040 (KS08/260)
Tula_virus_KS08_350_M	KS09/350	Germany: Gotha	*Microtus arvalis*	lung	M segment	MK535042	
Tula_virus_KS08_362_M	KS08/362	Germany: Gotha	*Microtus arvalis*	lung	M segment	MK535043	
Tula_virus_KS08_538_M	KS08/538	Germany: Gotha	*Microtus arvalis*	lung	M segment	MK535044	
Tula_virus_KS08_545_M	KS08/545	Germany: Gotha	*Microtus arvalis*	lung	M segment	MK535045	
Tula_virus_KS08_639_M	KS08/639	Germany: Westergellersen	*Microtus arvalis*	lung	M segment	MK535046	MK535047 (KS08/1045)
Tula_virus_KS08_1045_M	KS08/1045	Germany: Westergellersen	*Microtus arvalis*	lung	M segment	MK535047	MK535046 (KS08/639)
Tula_virus_KS09_1477_M	KS09/1477	Germany: Groß Schoenebeck	*Microtus arvalis*	lung	M segment	MK535048	
Tula_virus_KS09_1648_M	KS09/1648	Germany: Muckrow	*Microtus arvalis*	lung	M segment	MK535049	
Tula_virus_KS09_1657_M	KS09/1657	Germany: Huehnerwasser	*Microtus arvalis*	lung	M segment	MK535050	
Tula_virus_KS09_1669_M	KS09/1669	Germany: Huehnerwasser	*Microtus arvalis*	lung	M segment	MK535051	
Tula_virus_KS09_1886_M	KS09/1886	Germany: Huehnerwasser	*Microtus arvalis*	lung	M segment	MK535052	MK535053 (KS09/1912); MK535062 (KS10/905)
Tula_virus_KS09_1912_M	KS09/1912	Germany: Biebersdorf	*Microtus agrestis*	lung	M segment	MK535053	MK535052 (KS09/1886); MK535062 (KS10/905)

Table S2 (continued)

Sequence ID	Vole ID	Country/Site	Host	Isolation source	Note	Accession Numbers	Identical to
Tula_virus_KS09_1917_M	KS09/1917	Germany: Lohsa	*Microtus arvalis*	lung	M segment	MK535054	MK535055 (KS09/1939)
Tula_virus_KS09_1939_M	KS09/1939	Germany: Lohsa	*Microtus arvalis*	lung	M segment	MK535055	MK535054 (KS09/1917)
Tula_virus_KS09_2375_M	KS09/2375	Germany: Jeeser	*Microtus arvalis*	lung	M segment	MK535056	
Tula_virus_KS10_23_M	KS10/23	Germany: Jeeser	*Microtus arvalis*	lung	M segment	MK535057	MK535058 (KS10/29)
Tula_virus_KS10_29_M	KS10/29	Germany: Jeeser	*Microtus arvalis*	lung	M segment	MK535058	MK535057 (KS10/23)
Tula_virus_KS10_183_M	KS10/183	Germany: Ruethen	*Microtus arvalis*	lung	M segment	MK535059	MK535060 (KS10/185)
Tula_virus_KS10_185_M	KS10/185	Germany: Ruethen	*Microtus arvalis*	lung	M segment	MK535060	MK535059 (KS10/183)
Tula_virus_KS10_215_M	KS10/215	Germany: Soest	*Microtus arvalis*	lung	M segment	MK535061	
Tula_virus_KS10_905_M	KS10/905	Germany: Treben	*Microtus arvalis*	lung	M segment	MK535062	MK535052 (KS09/1886); MK535053 (KS09/1912)
Tula_virus_KS10_908_M	KS10/908	Germany: Treben	*Microtus arvalis*	lung	M segment	MK535063	MK535064 (KS10/932)
Tula_virus_KS10_932_M	KS10/932	Germany: Treben	*Microtus arvalis*	lung	M segment	MK535064	MK535063 (KS10/908)
Tula_virus_KS10_1188_M	KS10/1188	Germany: Treben	*Microtus agrestis*	lung	M segment	MK535065	
Tula_virus_KS10_1661_M	KS10/1661	Germany: Huehnerwasser	*Microtus arvalis*	lung	M segment	MK535066	
Tula_virus_KS11_1429_M	KS11/1429	Germany: Wolbrechtshausen	*Microtus arvalis*	lung	M segment	MK535067	

Table S2 (continued)

Sequence ID	Vole ID	Country/Site	Host	Isolation source	Note	Accession Numbers	Identical to
Tula_virus_KS13_784_M	KS13/784	Germany: Schrevendorf	*Microtus arvalis*	lung	M segment	MK535068	
Tula_virus_D34_L	D34	Germany: Berlin	*Microtus arvalis*	lung	L segment	MK535003	
Tula_virus_KS07_862_L	KS07/862	Germany: Tremt	*Microtus arvalis*	lung	L segment	MK535004	
Tulv_virus_KS08_260_L	KS08/260	Germany: Warburg	*Microtus arvalis*	lung	L segment	MK535005	
Tula_virus_KS08_277_L	KS08/277	Germany: Warburg	*Microtus arvalis*	lung	L segment	MK535006	MK535013 (KS09/1477)
Tula_virus_KS08_350_L	KS08/350	Germany: Gotha	*Microtus arvalis*	lung	L segment	MK535007	
Tula_virus_KS08_362_L	KS08/362	Germany: Gotha	*Microtus arvalis*	lung	L segment	MK535008	
Tula_virus_KS08_538_L	KS08/538	Germany: Gotha	*Microtus arvalis*	lung	L segment	MK535009	
Tula_virus_KS08_545_L	KS08/545	Germany: Gotha	*Microtus arvalis*	lung	L segment	MK535010	
Tula_virus_KS08_639_L	KS08/639	Germany: Westergellersen	*Microtus arvalis*	lung	L segment	MK535011	MK535012 (KS08/1045)
Tula_virus_KS08_1045_L	KS08/1045	Germany: Westergellersen	*Microtus arvalis*	lung	L segment	MK535012	MK535011 (KS08/639)
Tula_virus_KS09_1477_L	KS09/1477	Germany: Groß Schoenebeck	*Microtus arvalis*	lung	L segment	MK535013	MK535006 (KS08/277)
Tula_virus_KS09_1648_L	KS09/1648	Germany: Muckrow	*Microtus arvalis*	lung	L segment	MK535014	

Table S2 (continued)

Sequence ID	Vole ID	Country/Site	Host	Isolation source	Note	Accession Numbers	Identical to
Tula_virus_KS09_1657_L	KS09/1657	Germany: Huehnerwasser	*Microtus arvalis*	lung	L segment	MK535015	
Tula_virus_KS09_1669_L	KS09/1669	Germany: Huehnerwasser	*Microtus arvalis*	lung	L segment	MK535016	MK535031 (KS11/1429)
Tula_virus_KS09_1886_L	KS09/1886	Germany: Biebersdorf	*Microtus arvalis*	lung	L segment	MK535017	
Tula_virus_KS09_1912_L	KS09/1912	Germany: Biebersdorf	*Microtus agrestis*	lung	L segment	MK535018	
Tula_virus_KS09_1917_L	KS09/1917	Germany: Lohsa	*Microtus arvalis*	lung	L segment	MK535019	MK535020 (KS09/1939)
Tula_virus_KS09_1939_L	KS09/1939	Germany: Lohsa	*Microtus arvalis*	lung	L segment	MK535020	MK535019 (KS09/1917)
Tula_virus_KS09_2375_L	KS09/2375	Germany: Jeeser	*Microtus arvalis*	lung	L segment	MK535021	
Tula_virus_KS10_23_L	KS10/23	Germany: Jeeser	*Microtus arvalis*	lung	L segment	MK535022	
Tula_virus_KS10_29_L	KS10/29	Germany: Jeeser	*Microtus arvalis*	lung	L segment	MK535023	
Tula_virus_KS10_183_L	KS10/183	Germany: Ruethen	*Microtus arvalis*	lung	L segment	MK535024	MK535025 (KS10/185)
Tula_virus_KS10_185_L	KS10/185	Germany: Ruethen	*Microtus arvalis*	lung	L segment	MK535025	MK535024 (KS10/183)
Tula_virus_KS10_215_L	KS10/215	Germany: Soest	*Microtus arvalis*	lung	L segment	MK535026	MK535027 (KS10/905); MK535029 (KS10/932)
Tula_virus_KS10_905_L	KS10/905	Germany: Treben	*Microtus arvalis*	lung	L segment	MK535027	MK535026 (KS10/215); MK535029 (KS10/932)

Table S2 (continued)

Sequence ID	Vole ID	Country/Site	Host	Isolation source	Note	Accession Numbers	Identical to
Tula_virus_KS10_908_L	KS10/908	Germany: Treben	*Microtus arvalis*	lung	L segment	MK535028	
Tula_virus_KS10_932_L	KS10/932	Germany: Treben	*Microtus arvalis*	lung	L segment	MK535029	MK535026 (KS10/215); MK535027 (KS10/905)
Tula_virus_KS10_1188_L	KS10/1188	Germany: Treben	*Microtus agrestis*	lung	L segment	MK535030	
Tula_virus_KS11_1429_L	KS11/1429	Germany: Wolbrechtshausen	*Microtus arvalis*	lung	L segment	MK535031	MK535016 (KS09/1669)
Tula_virus_KS10_1661_L	KS10/1661	Germany: Huehnerwasser	*Microtus arvalis*	lung	L segment	MK535032	
Tula_virus_KS13_784_L	KS13/784	Germany: Schrevendorf	*Microtus arvalis*	lung	L segment	MK535033	
Tula_virus_Lodz_L	-	-	cell culture	cell culture	L segment	MK535037	

Table S3: **Pairwise comparison of partial nucleotide and amino acid sequences of the S segment, N protein, L segment and RNA-dependent RNA polymerase.** Pairwise comparison of partial nucleotide (nt) and amino acid (aa) sequences of the S segment (nucleotides 622-1015, numbering according to Khabarovsk orthohantavirus, GenBank accession number NC_034527) and the nucleocapsid (N) protein (amino acid residues 194-324) of the novel field vole-associated Traemmersee virus to the most related reference sequences identified by Basic Local Alignment Search Tool (BLAST) and of partial nt and aa sequences of the L segment (nucleotides 2962- 3324, numbering according to Khabarovsk orthohantavirus, GenBank accession number NC_034519) and the RNA-dependent RNA polymerase (RdRP; amino acid residues 976-1096). All sequences that have at least in either partial S or partial L segment nt sequence identities greater 75% are shown.

	S segment / N protein		L segment / RdRP	
Reference sequence	nt	aa	nt	aa
Tatenale_JX316009; JX316008	0.798	0.947	0.813	0.927
Kielder_KY751731	n.a.	n.a.	0.807	0.936
Kielder_KY751732	n.a.	n.a.	0.804	0.927
KHAV strain Topografov_AJ011646	0.789	0.85	n.a.	n.a.
KHAV_NC_034527, NC034519	0.771	0.843	0.765	0.918
Fusong_ FJ170792, KJ857316	0.766	0.835	0.765	0.9
Fusong_FJ170796	0.766	0.813	n.a.	n.a.
Fusong_NC_038446	0.766	0.82	n.a.	n.a.
Puumala_Muju_JX028273, JX046485	0.743	0.768	0.777	0.891
Puumala_Hokkaido_AB675474, AB712372	0.746	0.791	0.774	0.855

Table S3 (continued)

Reference sequence	S segment / N protein		L segment / RdRP	
	nt	aa	nt	aa
PUUV_Ussuri_Khekhtsir_AB677476	0.746	0.783	n.a.	n.a.
PUUV_like_Fusong_ EF211820	0.756	0.791	n.a.	n.a.
Fugong_ KT899701, KT899703	0.606	0.597	0.768	0.81
Bayou_DQ256126, FJ858378	0.559	0.537	0.753	0.837
Prospect_Hill_ NC_038938, EF646763	0.686	0.716	0.753	0.882
Puumala_NC_005224, NC_005225	0.738	0.765	0.76	0.873
Tula_NC_005227, 005226	0.611	0.651	0.756	0.864

n.a., not available

Table S4: Primers used for hantavirus RT-PCR screening, RT-PCR amplification of M and L segment sequences of Tula orthohantavirus, and primer-walking based determination of the complete coding S, M and L segment sequences of the novel field vole-associated Traemmersee virus.

Segment	Name	Sequence 5´->3´	Used annealing Temperature °C	Starting-Position of Primer*	Reference
S	PU342F	TATGGTAAATGTCCTTGATGT	46.2	S 334	[1]
S	PU1102R	GCCATDATDGTRTTYCTCAT	46.2	S 1066	[1]
S	TraeSFor1	GTAGTAGACTCCTTGARRAGCTACTAC	58	S 3	this study
S	TraeSREV1	CTACCCGTGTCGAAAGCAT	58	S 460	this study
S	SFOR_A2	GAGCTCGGGGCATTCTTTC	58	S 1033	this study
S	SREV_A1	GATAGCTCAGTTCACATTNDYGG	58	S 1823	this study
L	LFOR_A1	TAGTAGTAGACTCCGAGAKAGAGAA	58	L 1	this study
L	L190F	TCTTACTGATGGCAGGTGTGC	58	L 215	this study
L	L315R	CCT GTT ATY TTR TAR TTR TCT GGT GTC	58	L 318	this study
L	L658R	CTG TDA CAT GRA AYT TYA AAT TRA ACA TDG C	58	L 661	this study
L	L650_2F	GAWGAGAGRGSRGCAYTWGAAG	58	L640	this study
L	L1505F	CWG GKY TTA AAA GAT CWA ART AYT GGT C	58	L 1505	this study
L	L1665R	CCT AAC ACC ATC TAT TTC AAC CTT TG	58	L 1670	this study
L	L1946F	ACTCTGGCTATGGACCCTGA	58	L1946	this study
L	L2124RMOD	GAR GRA TAM AYC CCR CTW GCT C	58	L2123	this study
L	L2379F	GCAACAGCTCTTTTGTCAGGA	55	L 2379	this study
L	L2549R	GCCGATAAATGCCCATCCTCT	58	L 2550	this study
L	L3040R	GCATCCACCACGCAGTTTTT	55	L 3040	this study
L	HANLF1	ATGTAYGTBAGTGCWGATGC	51.2	L 2935	[2]
L	HANLR1	AACCADTCWGTYCCRTCATC	51.2	L 3367	[2]
L	LFOR935x	GGTAATTGGCTACAGGGTAA	58	L 3196	this study
L	LREV935x	CACCACCYAATGGKATAGG	58	L 3820	this study
L	L3700F	CACAATTGGCACAACTAGGGA	55	L 3701	this study

Table S4 (continued)

Segment	Name	Sequence 5´->3´	Used annealing Temperature °C	Starting-Position of Primer*	Reference
L	L4250FMOD	RGTTCAGRATGCAGGDGAARCARCA	56	L 4251	this study
L	L4483F	CGG CCT AAA ATT GCG AGG AC	57	L 4483	this study
L	L4935R	CYT TRC ACC AYC TCC AYA YYT C	58	L 4936	this study
L	L5365R	CTT GTT CWG TYT TRA ATC TCA TNC CRC	58	L 5366	this study
L	L5365F	GYG GNA TGA GAT TYA ARA CWG AAC AAG	58	L 5366	this study
L	L5211F	TGAAGGCGATCTTCGTGTTACT	56	L 5211	this study
L	L5432R	TTTCCCCCACTCAAAGCCAC	57	L 5432	this study
L	L5433F	GGCTTTTAGTGGGGTAAGCCT	56	L 5432	this study
L	L5605F	CAC ATG CTT ATC ACA ATC TAA AGG AC	58	L 5605	this study
L	L5865RMOD	TGT YTC YTC AAT CCC ATC CAR RTT	57	L 5863	this study
L	LENDREV	GTA GTA TGC TCC GTG AAA AGA GCA ATA	58	L 6522	this study
M	MFORC1	TAGTAGTAGAYTCCGCAAGAARGAA	58	M 1	this study
M	MF590	GTGTATCWGGNCARCTTRTWGAAG	58	M 588	this study
M	MR669	CAGTGCACAGGCAATGTTACAA	58	M 672	this study
M	MF1000	DTNCAAGGKRTWGCATT	56	M 998	this study
M	MFOR1320	GGKGCWGARCARCARRTHAARTTTG	58	M 1310	this study
M	MR1410	GGGAAAATATGTCTAGTAAAAGTATATATG	56	M 1423	this study
M	MFA1	ATGGGYTCWATGGTWTGTGARGTHT	53	M 1690	this study
M	MREV1750	GTTCCATTTGGGCAGCTCTTTTATG	58	M 1748	this study
M	MR2400	ACA ACC TGT ACC TAC CCC TG	53	M 2379	this study
M	C1m	CCAGCTGATTGCCCAGGGGTAG	56.4	M 2366	[3]
M	C2m	CCTACTCCTGAGCCCCATGC	56.4	M 3008	[3]
M	TraeMFOREnd	CAGAACTTCTATTGATGGAG	48	M 2989	this study
M	Mrev4.2 mod	AAAAGTCCAGGCATRATCG	48	M 3666	this study
M	MEN1FOR	ACAGGDTGTACWGCWTGTGGTG	58	M 2393	this study
M	MEN1REV	AGCTRTCWGGGTCTACCCAYTC	58	M 2885	this study

Table S4 (continued)

Segment	Name	Sequence 5´->3´	Used annealing Temperature °C	Starting-Position of Primer*	Reference
M	MCN2FOR	GGRACWGAACAAACATGYAAAACNATWGA	58	M 2504	this study
M	MCN1REV	TCYTTTARTGARCTRTCAGGRTCYAC	58	M 2891	this study
L	LEN2FOR	ACAAAGTGGTCACCTGGTGATAATCA	58	L 2957	this study
L	LEN1REV	CATCATCAGAGTGGTGTGCAAATTC	58	L 3310	this study
L	LCN1FOR	ACAAARTGGTCACCWGGTGATAAYTCA	58	L 2656	this study
L	LCN1REV	GCATCATCAGARTGRTGTGCAAAYTC	58	L 3310	this study

* Numbering according to position in S (NC_034527), L (NC_034519) and M segment (NC_034518) of Khabarovsk orthohantavirus reference sequence and for TULV lineage specific primers according to Tula orthohantavirus reference sequence for M (NC_005228) and L segment (NC_005226).

Table S5: Pairwise comparison of complete coding nucleotide (nt) sequences and amino acid (aa) sequences of the entire nucleocapsid (N) protein, glycoprotein precursor (GPC) protein, and RNA-dependent RNA polymerase (RdRP) of the novel field vole-associated Traemmersee virus to the most related reference sequences and pairwise evolutionary distance (PED) values.

	S / N protein		M / GPC		L / RdRP		PED
Reference sequence*	nt	aa	nt	aa	nt	aa	
Fugong_ KT899701, EU072488	0.747	0.761	0.696	0.716	n.a.	n.a.	0.33118
Fusong_NC_038446, NC_038447, KJ857316	0.8	0.882	0.75	0.85	0.778	0.911	0.15486
Puumala_NC_005224, NC_005223, NC_005225	0.764	0.865	0.748	0.821	0.821	0.884	0.18071
Khabarovsk_NC_034527, NC_034518, NC_034519	0.791	0.884	0.748	0.861	0.778	0.904	0.14312
Yakeshi NC_038704, EU072489	0.629	0.623	0.758	0.857	n.a.	n.a.	0.66441
Tula_NC_005227, NC_005228, NC_005226	0.735	0.803	0.739	0.817	0.761	0.87	0.21063

n.a., not available; *, Reference sequences that reached a nucleotide sequence identity of at least 75% in one of the three segments were selected.

Table S6: Accession numbers for all hantavirus sequences used in Fig. 1 and Fig. S3.

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Altai hantavirus				KM361055-KM361057	KM361055-KM361057
Amga orthohantavirus	KF974360- KM201411	KF974360- KM201411			
Andes orthohantavirus	AF004660, AF325966, AF482713-AF482717, AY228237, DQ345763, EF571895, KY659432, NC_003466, KP202360	AF004660, AF325966, AF482713-AF482717, AY228237, DQ345763, EF571895, KY659432, NC_003466, KP202360	AF324901, AF291703, AY228238, KY604962	AF291704, JX443698	AF291704, JX443698
Asama orthohantavirus	EU929070-EU929071	EU929070-EU929071	EU929073-EU929075		
Asikkala orthohantavirus	KC880341-KC880343	KC880341-KC880343	KC880344-KC880345		
Bayou orthohantavirus	NC_038298	NC_038298	GQ244521, L36930	FJ858378, GQ244526	FJ858378, GQ244526
Black Creek Canal orthohantavirus	L39949	L39949		GU997097	GU997097
Bowe orthohantavirus				KC631784	KC631784
Bruges orthohantavirus	MF683844	MF683844		KX551962	KX551962
Camp Ripley hantavirus				KF958465	KF958465
Cano Delgadito orthohantavirus	NC_034528	NC_034528		GQ200821	GQ200821
Cao Bang orthohantavirus	JX465405- JX465406, JX465409-JX465410, KJ162406, NC_034484		KJ162397, NC_034474	EF543525, KF705679	EF543525, KF705679
Choclo orthohantavirus	DQ285046, KM597161, KT983771	DQ285046, KM597161, KT983771	DQ285047, KT983772	EF397003, KT983773	EF397003, KT983773

Table S6 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Dobrava-Belgrade orthohantavirus	AJ009773-AJ009775, AJ269549-AJ269550, AJ131672-AJ131673, AJ410619, AY168576, AY961618, AY533120, EU188449-EU188452, GQ205401-GQ205402, GQ205404-GQ205407, GU904027-GU904028, JF920150, JQ026204, KT885043, NC_005233	AJ009773-AJ009775, AJ269549-AJ269550, AJ131672-AJ131673, AJ410619, AY168576, AY961618, AY533120, EU188449-EU188452, GQ205401-GQ205402, GQ205404-GQ205407, GU904027-GU904028, JF920150, JQ026204, KT885043, NC_005233	AJ009774, AY168578, AY961616, GQ205411, GU904033-GU904034, KT885042, L33685, Q205409, Y168577	AJ410617, JF920148, JQ026206	AJ410617, JF920148, JQ026206
EL Moro Canyon orthohantavirus	NC_034396	NC_034396			
Fugong orthohantavirus	KT899701	KT899701	NC_034466		
Fusong orthohantavirus	EU072480-EU072481, FJ170792,FJ170796, FJ170797:	EU072480-EU072481, FJ170792,FJ170796, FJ170797		KJ857316	KJ857316
Hantaan orthohantavirus	AF427318-AF427319, AF427322-AF427323, KJ857347-KJ857348	AF427318-AF427319, AF427322-AF427323, KJ857347-KJ857348	AB027115, EU363815, FJ753399, JQ665881-JQ665882, JQ912804, KP970560, KU207203-KU207204, L08753, Y00386	X55901, AB620030, AF189155, AF285266, AF288297, AF293665, AF288292, AY675354, D25531, DQ056292, KC136242, KJ857317	X55901, AB620030, AF189155, AF285266, AF288297, AF293665, AF288292, AY675354, D25528, D25531, DQ056292, KC136242, KJ857317
Imjin thottimvirus	EF641804-EF641805, KJ420559	EF641804-EF641805, KJ420559	EF641797 - EF641799, KX779127, NC_034557		
Isla Vista hantavirus	U19302, U31534-U31535	U19302, U31534-U31535			
Jeju orthohantavirus	HQ663933, HQ834695	HQ663933, HQ834695		HQ663935, HQ834697	HQ663935, HQ834697
Kenkeme orthohantavirus	GQ306148, MG279218	GQ306148, MG279218		KJ857320	KJ857320

Table S6 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Khabarovsk orthohantavirus	AJ011646, KJ857343-KJ857346, NC_034527	AJ011646, KJ857343-KJ857346, NC_034527	AJ011648, EU072489, KJ857330, KJ85733, NC_034518	KJ857311- KJ857315, KJ857321-KJ857322	KJ857311- KJ857315, KJ857321-KJ857322
Kielder hantavirus				KY751731-KY751732	KY751731-KY751732
Kilimanjaro hantavirus				JX193697	JX193697
Marporal orthohantavirus				EU788002	EU788002
Laguna Negra orthohantavirus	AF005727, KP202359	AF005727, KP202359		FJ809772, JQ6111713, JX443696- JX443697	FJ809772, JQ6111713, JX443696- JX443697
Laibin mobatvirus	KM102247, KY662264	KM102247, KY662264	KM102248, KY662268-KY662269		
Longquan loanvirus	JX465413	JX465413	JX465397, JX465399-JX465400		
Luxi orthohantavirus				HQ404253, KT899703	HQ404253, KT899703
Montano orthohantavirus	NC_034396	NC_034396		AB620102, AB620105, AB620108	AB620102, AB620105, AB620108
Nova mobatvirus	KT004445, KX512328, KX512330, KX512345	KT004445, KX512328, KX512330, KX512345	KT004446, KX512426-KX512430, KY780087, NC_034470		
Necocli orthohantavirus	KF481954	KF481954			
Oxbow orthohantavirus	FJ539166	FJ539166			
Prospect Hill orthohantavirus	NC_038938, M34011, U47136	NC_038938, M34011, U47136		EF646763	EF646763

Table S6 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Puumala orthohantavirus	AB297665, AB675463, AB675474, AB675478, AB677476-AB677477, AF367064-AF367066, AF367068, AF367069-AF367071, AF442613, AJ223368- AJ223369, AJ223371, AJ223374-AJ223375, AJ223376-AJ223377, AJ223380, AY526219, DQ138140, DQ138142, EF211819-EF211820, EF442087, GQ339473, GQ339477-GQ339481, GQ339483-GQ339484, GQ339487, JN657228, KT885052, M32750, U14137, Z21497, Z30706-Z30708, Z84204	AB297665, AB675463, AB675474, AB675478, AB677476-AB677477, AF367064-AF367066, AF367068, AF367069-AF367071, AF442613, AJ223368- AJ223369, AJ223371, AJ223374-AJ223375, AJ223376-AJ223377, AJ223380, AY526219, DQ138140, DQ138142, EF211819-EF211820, EF211820, EF442087, GQ339473, GQ339477- GQ339481, GQ339483-GQ339484, GQ339487, JN657228, JX028273, KT885052, M32750, U14137, Z21497, Z30706-Z30708, Z84204	AB297666, AB433850-AB433852, AF442615-AF442616, AY526218, EF422372, HE801634, JN831944, JQ319172, JQ319174-JQ319175, L08755, M29979, NC_005223, Z84205, Z49214	AB297667, AB574183-AB574184, AB712372, AY526217, EF405801, HE801635, JN831946, JN831949, JN831952, JX028271, JX046482-JX046485, KJ994778, KT885050, M63194	AB297667, AB574183-AB574184, AB712372, AY526217, EF405801, HE801635, JN831946, JN831949, JN831952, JX028271, JX046482-JX046485, KJ994778, KT885050, M63194
Quezon mobatvirus	KU950713	KU950713			
Rockport orthohantavirus	HM015218, HM015223-HM015224	HM015218, HM015223-HM015224		HM015220-HM015222	HM015220-HM015222
Sangassou orthohantavirus	JQ082303, NC_034526	JQ082303, NC_034526			

Table S6 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Seoul orthohantavirus	AF288653, AF288655, EF192308, FJ803205-FJ803208, FJ803210-FJ803211, FJ803213-FJ803216, GQ274945, GQ279392, GU592938, GU592941-GU592944, GU592946-GU592949, GU592951, GU592953, GU361893, JQ898106, KF387725, KM948598, KP859511, MF149948	AF288653, AF288655, EF192308, FJ803205-FJ803208, FJ803210-FJ803211, FJ803213-FJ803216, GQ274945, GQ279392, GU592938, GU592941-GU592944, GU592946-GU592949, GU592951, GU592953, GU361893, JQ665912, JQ898106, KF387725, KM948598, KP859511, MF149948	AF145977, D17592, GU592924-GU592926, GU592929, GQ274943, JQ665888, JX879768, MF149945, N377554, X53861		
Sin Nombre orthohantavirus	JQ690277-JQ690278, JQ690282, KT885046, L37904, NC_005216	JQ690277-JQ690278, JQ690282, KT885046, L37904, NC_005216	AF030551-AF030552, JQ690279-JQ690280, JQ690283-JQ690284, KT885045, L37903, NC_005215	AF425256, KT885044, L37901-L37902, MG717393, MH539865	AF425256, KT885044, L37901- L37902, MG717393; MH539865
Tatenale hantavirus	JX316009	JX316009		JX316008	JX316008
Thailand orthohantavirus	AB186420, AM397664, AM998808, KC490915-KC490916, KC490918	AB186420, AM397664, AM998808, KC490915-KC490916, KC490918		KC490922, KU587796	KC490922, KU587796
Tigray orthohantavirus				KU934008	KU934008
Thottapalayam thottimvirus	JF784172, KJ420560	JF784172, KJ420560	DQ825771, EU001329 JF784178- JF784180, KJ420542- KJ420545		
Traemmersee orthohantavirus	MK542662	MK542662	MK542663	MK542664	MK542664

Table S6 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Tula orthohantavirus	AF017659, AF063892, AF164093-AF164094, AF289819-AF289821, AF442621, AJ223600-AJ223601, AM945877, EU439949-EU439951, KT946591, KU139529-KU139531, KU139533, KU139535, KU139537-KU139539, KU139549, KU139553, KU139555-KU139557, KU139558, KU139565-KU139566, KU139569-KU139570, KU139577-KU139579, KU139595-KU139597, KU139600, KU139605, MK386130-MK386141 MK535072, MK535084, MK535086, NC_005227, Y13979-Y13980, Z30941-Z30945, Z48741-Z48574, Z69991	AF017659, AF063892, AF164093-AF164094, AF289819-AF289821, AF442621, AJ223600-AJ223601, AM945877, EU439949-EU439951, KT946591, KU139529-KU139531, KU139533, KU139535, KU139537-KU139539, KU139549, KU139553, KU139555-KU139557, KU139558, KU139563, KU139565-KU139566, KU139569-KU139570, KU139577-KU139579, KU139595-KU139597, KU139600, KU139605, MK386130-MK386141 MK535072, NC_005227, Y13979-Y13980, Z30941-Z30945, Z48741-Z48574, Z69991	NC_005228	AJ005637, MK386130-MK386140, MK535003-MK535005, MK535007-MK535011, MK535013-MK535018, MK535020-MK535024, MK535027-MK535029, MK535030, MK535032-MK535037	
Uluguru hantavirus				JX193700	JX193700
Xinyi				KF705679	KF705679
Xuan Son hantavirus			KY662265-KY662271	KY662269, KY662272, KY662275	KY662269, KY662272, KY662275
Yakeshi orthohantavirus	NC_038704	NC_038704			

References

1. Essbauer S, Schmidt J, Conraths FJ, Friedrich R, Koch J, Hautmann W, Pfeffer M, Wolfel R, Finke J, Dobler G, Ulrich R (2006) A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. Epidemiology and infection 134 (6):1333-1344. doi:10.1017/s0950268806006170

2. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Denys C, Koivogui L, ter Meulen J, Kruger DH (2006) Hantavirus in African wood mouse, Guinea. Emerging infectious diseases 12 (5):838-840. doi:10.3201/eid1205.051487

3. Mertens M, Kindler E, Emmerich P, Esser J, Wagner-Wiening C, Wolfel R, Petraityte-Burneikiene R, Schmidt-Chanasit J, Zvirbliene A, Groschup MH, Dobler G, Pfeffer M, Heckel G, Ulrich RG, Essbauer SS (2011) Phylogenetic analysis of Puumala virus subtype Bavaria, characterization and diagnostic use of its recombinant nucleocapsid protein. Virus genes 43 (2):177-191. doi:10.1007/s11262-011-0620-x

Supplementary material

Fig. S1:Phylogenetic trees of Tula orthohantavirus (TULV) partial S and M segment sequences. The trees are based on Maximum-Likelihood analysis with 1,000 bootstraps and 50% cut-off using the General Time Reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter of partial Ssegment (A) and partial M segment (B). Length of the S segment alignment is 639 nucleotides (nt), positions 379-1002, counting according TULV S segment, accession number NC_005227) (A) andlength of the M segment alignment is 348 nt, positions 2535-2884, counting according TULV M segment, accession number NC_005228) (B).Bootstrap support values greater than 70 are given at the supported nodes. Puumalaorthohantavirusreference sequences (NC 005224 and NC 005223) and Traemmerseevirus sequences (MK542662 and MK542663) were used as outgroup. Alignments were constructed under Bioedit (V7.2.3.) [38] using the Clustal W Multiple Alignment algorithm implemented in the program.The tree reconstructions were done via CIPRES [37]. The clade names TULV Central North (TULV-CEN.N), Eastern North (TULV-EST.N), Central South (TULV-CEN.S) and Eastern South (TULV-EST.S) follow previous classification [19]. Names in bold indicate new sequences and grey boxes indicate sequences from voles that were trapped in this study. Additional S and M segment sequences were generated for TULV strains belonging to the clades CEN.N and EST.N (for details see Table S2) using lineage specific primers (Table S4). Identical sequences were excluded from the analysis (see Table S2).

Fig. S2: Phylogenetic trees of partial cytochrome *b* gene sequences of common voles (*Microtus arvalis*) and field voles (*Microtus agrestis*). The phylogenetic analyses are based on Maximum-Likelihood analysis with 1,000 bootstraps and 50% cut-off using the General Time Reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter.The length of the alignment for common voles (*Microtus arvalis*) was 873 nt; nt positions 136-1008, counting according to *Microtus arvalis* isolate MA324 cytochrome *b*, accession number GU190526, nomenclature after [1-3](A). The length of the alignment for field voles (*Microtus agrestis*) was 895 nt; nt positions 114-1008, counting according to *Microtus agrestis* voucher D29 cytochrome *b*, accession number GU563294,nomenclature after [34](B). Bootstrap support values greater than 70 are given at the supported nodes.Alignments were

constructed under Bioedit (V7.2.3.) [38] using the Clustal W Multiple Alignment algorithm implemented in the program.The tree computations were done via CIPRES [37] and new sequences are written in bold. MK535091 is identical with MK535087. TULV-RNA and anti-TULV antibody (Ab) positive common voles (A) and field voles (B) are given in bold letters. TRAV positive field vole is underlined.

Fig. S3:Consensus phylogenetic trees of Traemmersee virus and other hantaviruses for S, M and L segment nucleotide sequences and corresponding amino acid sequences. The phylogenetic analyses for complete coding region of S (A), L (C) and M (E) are based on Bayesian analyses with 10,000,000 generations and a burn-in of 25%, and Maximum-Likelihood analysis with 1,000 bootstraps and 50% cut-off using the General Time Reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter segment (length of alignments: S:1359 nt, M: 3513 nt, L: 6516 nt). Posterior probabilities in percent from Bayesian analyses are given before the slash and bootstrap values are given after the slash for major nodes when they exceeded 70. Phylogenetic trees of amino acid sequences based on the Jones-Taylor-Thornton substitution model (JTT) with 20 numbers of rate categories per site (CAT) analysis with 1,000 bootstraps and 50% cut-off for nucleocapsid protein (N) (B), RNA-dependent RNA polymerase (RdRP) (D), and glycoprotein precursor protein (GPC) (F). The lengths of the alignments were 453 aa (N), 2172 aa (RdRP) and 1171aa(GPC). Bootstrap support values greater than 70 are given at the supported nodes.Alignments were constructed under Bioedit (V7.2.3.) [38] using the Clustal W Multiple Alignment algorithm implemented in the program.All tree reconstructions were done via CIPRES [37]. Bold written sequences indicate the new Traemmerseeorthohantavirus sequence. Triangles indicate condensed branches. Additional GenBank accession numbers are given in Table S6.

References

1. Martínková N, Barnett R, Cucchi T, Struchen R, Pascal M, Pascal M, Fischer MC, Higham T, Brace S, Ho SY (2013) Divergent evolutionary processes associated with colonization of offshore islands. Molecular ecology 22 (20):5205-5220. doi:10.1111/mec.12462

2. Stojak J, McDevitt AD, Herman JS, Kryštufek B, Uhlíková J, Purger JJ, Lavrenchenko LA, Searle JB, Wójcik JM (2016) Between the Balkans and the Baltic: phylogeography

of a common vole mitochondrial DNA lineage limited to Central Europe. PloS one 11 (12):e0168621. doi:10.1371/journal.pone.0168621

3. Heckel G, Burri R, Fink S, Desmet JF, Excoffier L (2005) Genetic structure and colonization processes in European populations of the common vole, Microtus arvalis. Evolution 59 (10):2231-2242
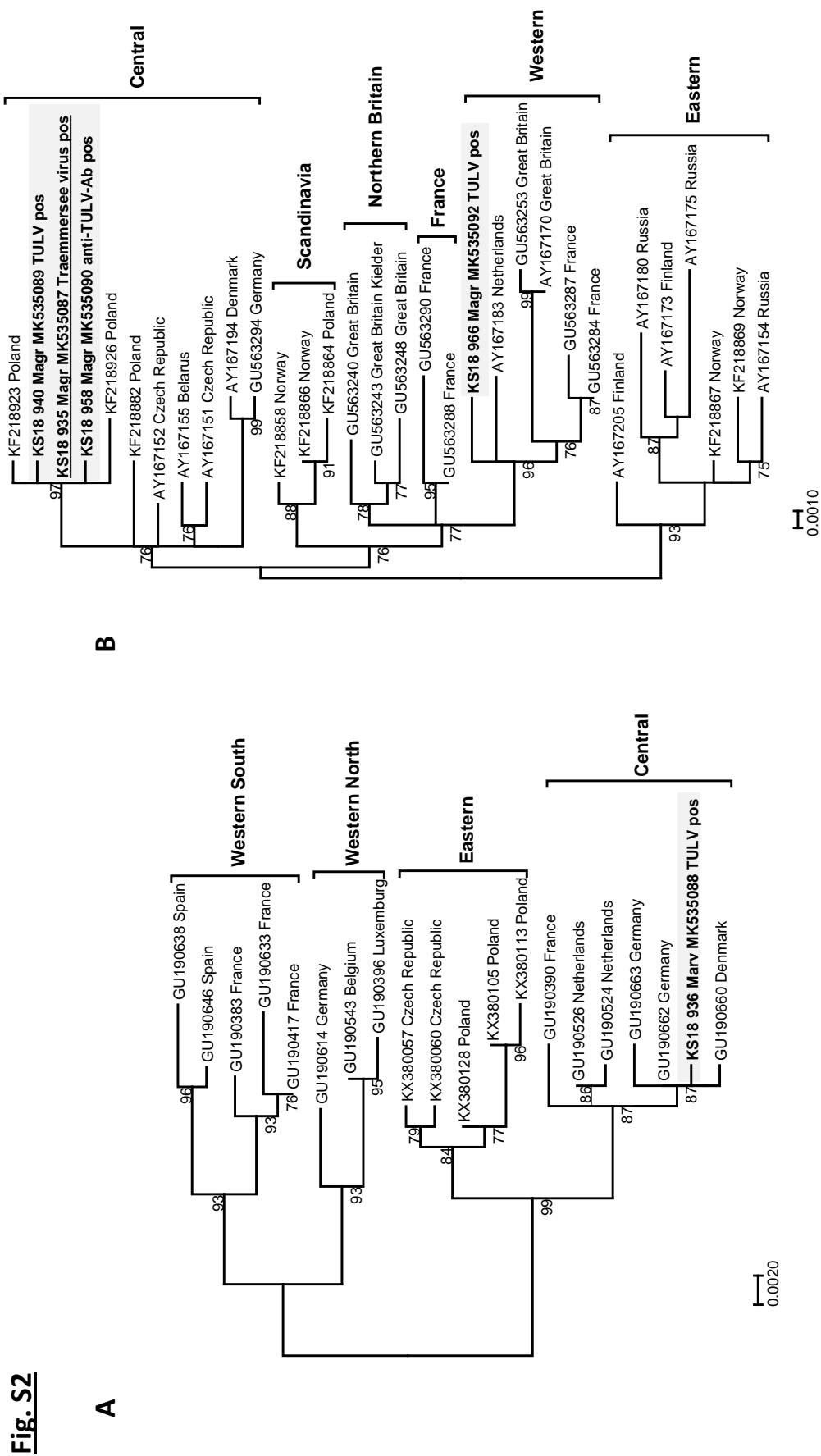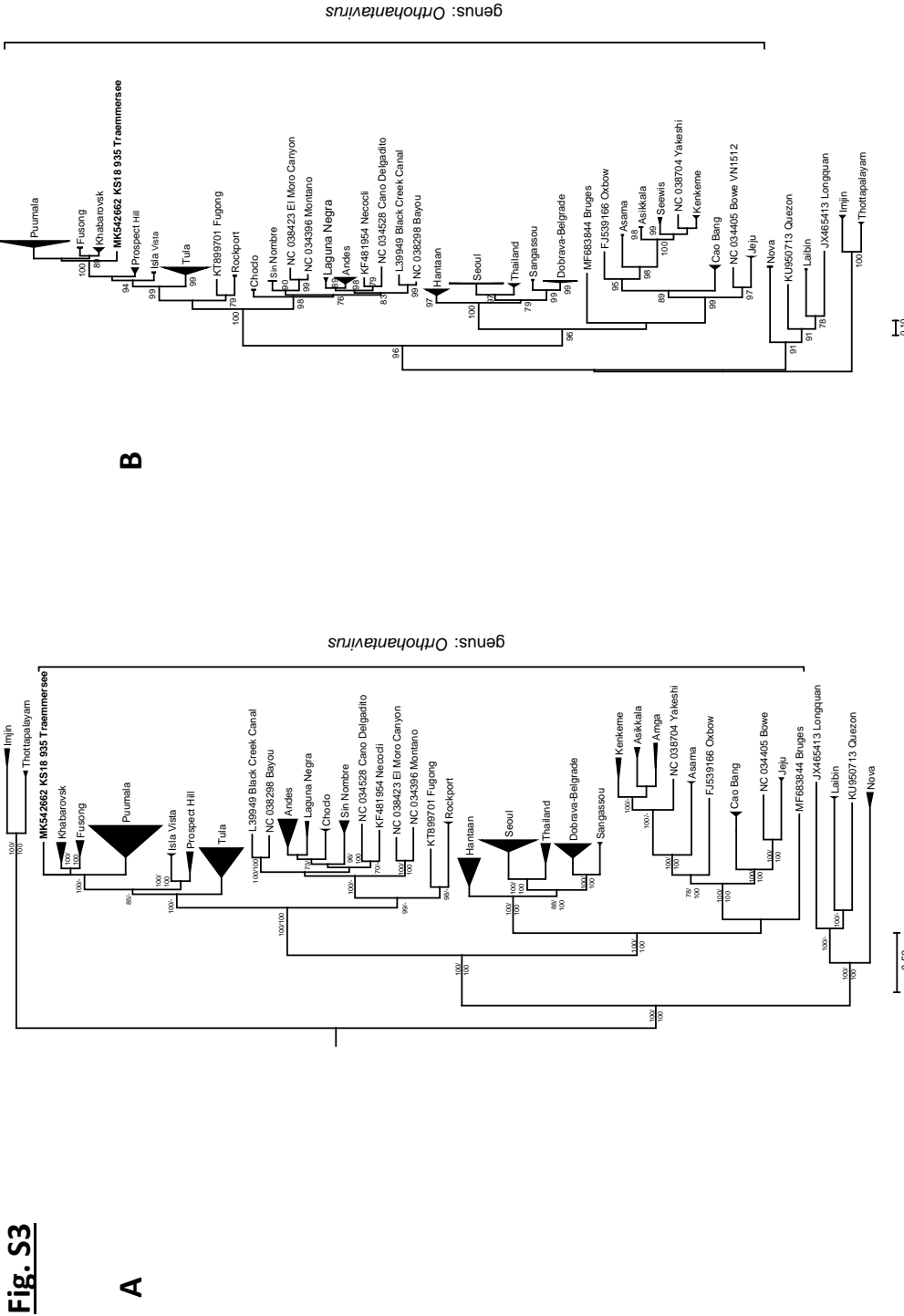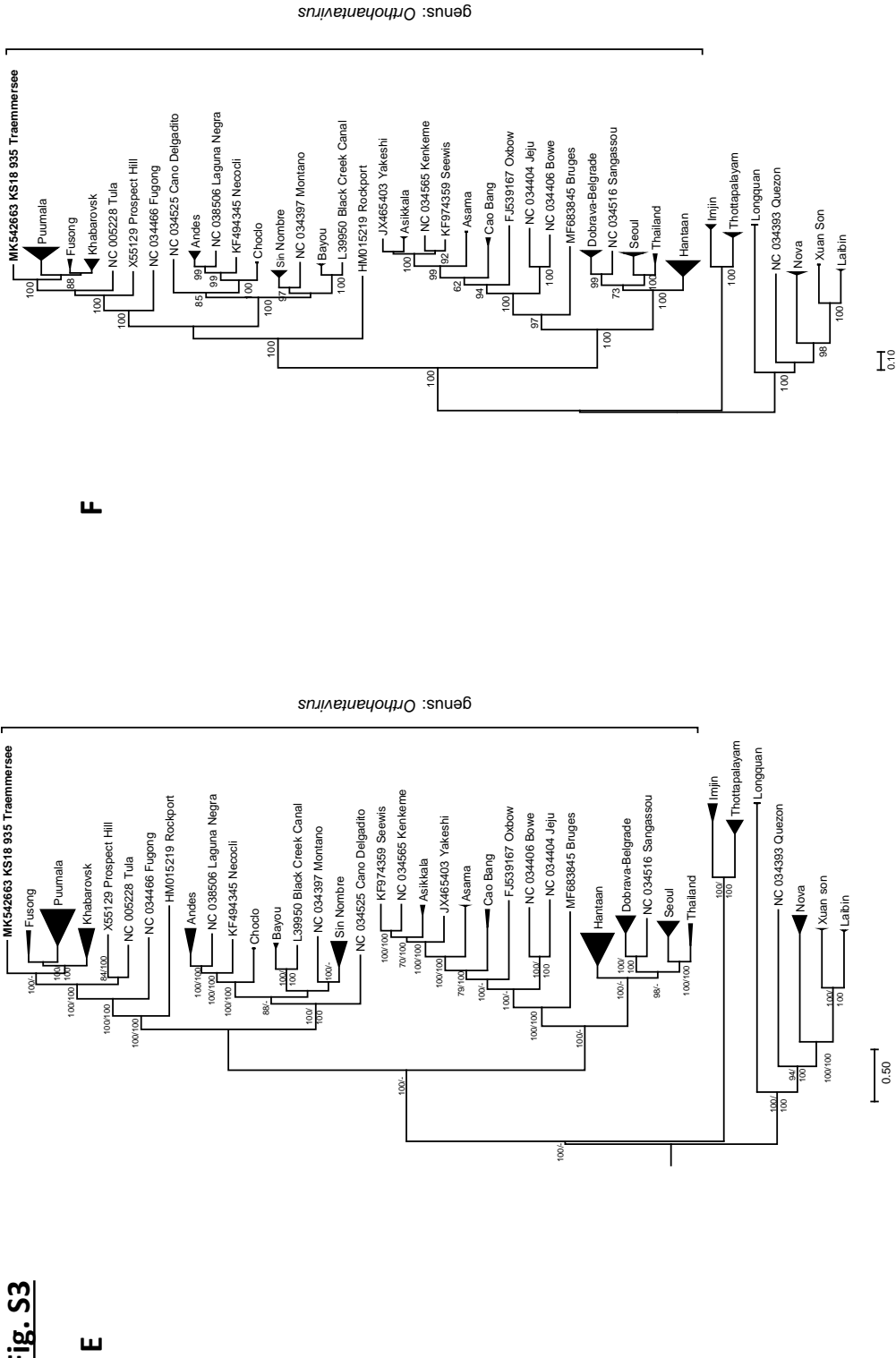
Fig. S1

Fig. S2

Fig. S3

A

genus: *Orthohantavirus*

B

genus: *Orthohantavirus*
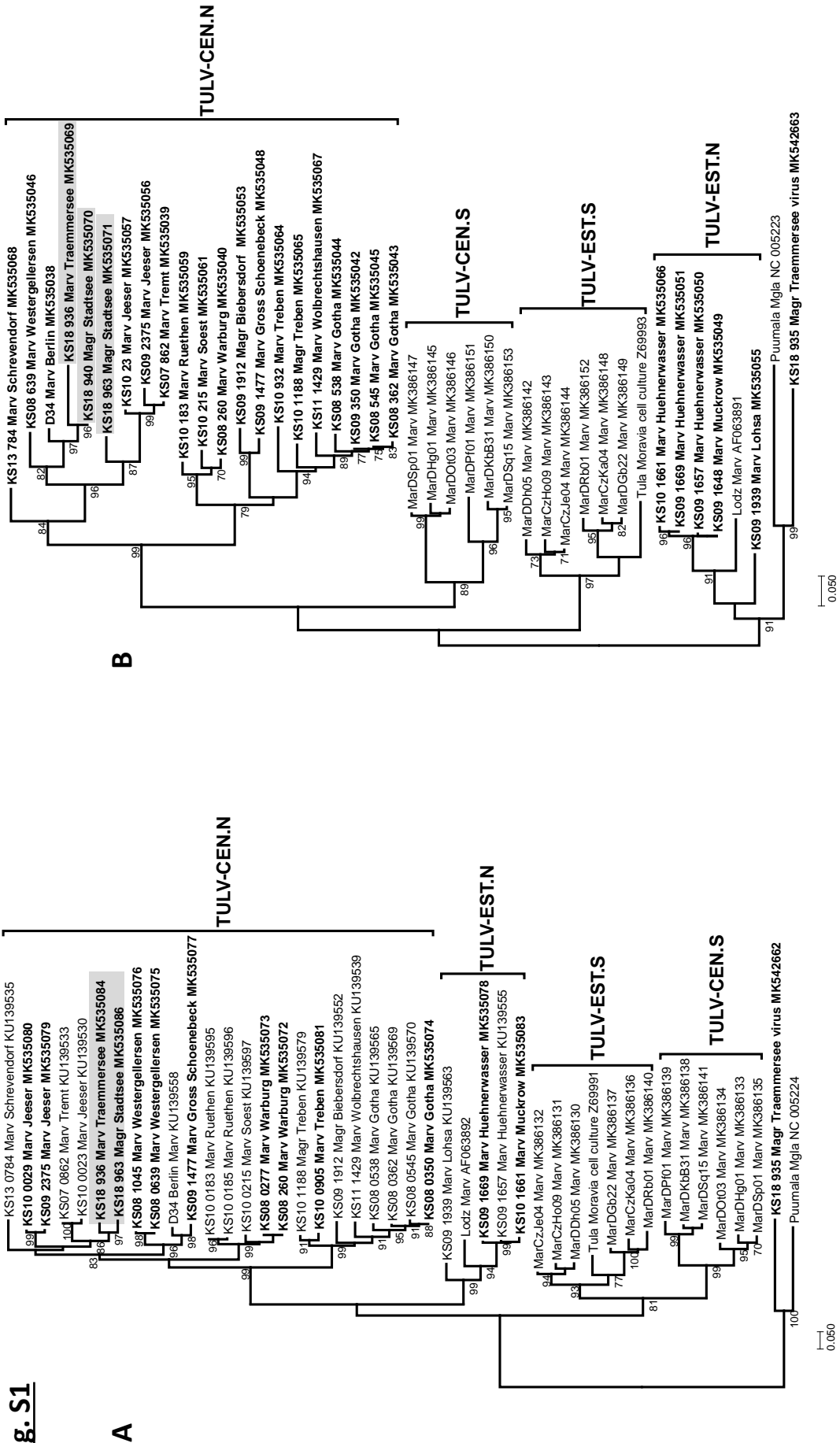
Fig. S3

Fig. S3

3.2 Paper II

Drewes S*, **Jeske K***, Straková P, Balčiauskas L, Ryll R, Balčiauskienė L, Kohlhause D, Schnidrig G, Hiltbrunner M, Špakova A, Insodaitė R, Petraitytė-Burneikienė R, Heckel G, Ulrich RG, (2020) Identification of a novel hantavirus strain in the root vole (*Microtus oeconomus*) in Lithuania, Eastern Europe. Infection, Genetics and Evolution. https://doi.org/10.1016/j.meegid.2020.104520

*both authors contributed equally

Research Paper

Identification of a novel hantavirus strain in the root vole (*Microtus oeconomus*) in Lithuania, Eastern Europe

Stephan Drewes [a,1], Kathrin Jeske [a,b,1], Petra Straková [a,c], Linas Balčiauskas [d], René Ryll [a], Laima Balčiauskienė [d], David Kohlhause [a,e], Guy-Alain Schnidrig [f], Melanie Hiltbrunner [f], Aliona Špakova [g], Rasa Insodaitė [g], Rasa Petraitytė-Burneikienė [g], Gerald Heckel [f], Rainer G. Ulrich [a,*]

[a] *Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany*
[b] *Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany*
[c] *Department of Virology, Veterinary Research Institute, Hudcova 70, 62100 Brno, Czech Republic*
[d] *Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania*
[e] *University Greifswald, Domstraße 11, 17498 Greifswald, Germany*
[f] *Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland*
[g] *Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio al. 7, LT-10257 Vilnius, Lithuania*

ARTICLE INFO

ABSTRACT

Hantaviruses are zoonotic pathogens that can cause subclinical to lethal infections in humans. In Europe, five orthohantaviruses are present in rodents: *Myodes*-associated Puumala orthohantavirus (PUUV), *Microtus*-associated Tula orthohantavirus, Traemmersee hantavirus (TRAV)/ Tatenale hantavirus (TATV)/ Kielder hantavirus, rat-borne Seoul orthohantavirus, and *Apodemus*-associated Dobrava-Belgrade orthohantavirus (DOBV). Human PUUV and DOBV infections were detected previously in Lithuania, but the presence of *Microtus*-associated hantaviruses is not known. For this study we screened 234 *Microtus* voles, including root voles (*Microtus oeconomus*), field voles (*Microtus agrestis*) and common voles (*Microtus arvalis*) from Lithuania for hantavirus infections. This initial screening was based on reverse transcription-polymerase chain reaction (RT-PCR) targeting the S segment and serological analysis. A novel hantavirus was detected in eight of 79 root voles tentatively named "Rusne virus" according to the capture location and complete genome sequences were determined. In the coding regions of all three genome segments, Rusne virus showed high sequence similarity to TRAV and TATV and clustered with Kielder hantavirus in phylogenetic analyses of partial S and L segment sequences. Pairwise evolutionary distance analysis confirmed Rusne virus as a strain of the species TRAV/TATV. Moreover, we synthesized the entire nucleocapsid (N) protein of Rusne virus in *Saccharomyces cerevisiae*. We observed cross-reactivity of antibodies raised against other hantaviruses, including PUUV, with this new N protein. ELISA investigation of all 234 voles detected Rusne virus-reactive antibodies exclusively in four of 79 root voles, all being also RNA positive, but not in any other vole species. In conclusion, the detection of Rusne virus RNA in multiple root voles at the same trapping site during three years and its absence in sympatric field voles suggests root voles as the reservoir host of this novel virus. Future investigations should evaluate host association of TRAV, TATV, Kielder virus and the novel Rusne virus and their evolutionary relationships.

1. Introduction

The genus *Orthohantavirus* belongs to the family *Hantaviridae* within the order *Bunyavirales* and currently contains 36 virus species (Schmaljohn and Dalrymple, 1983; ICTV, 2020). These segmented, negative strand RNA viruses are believed to have coevolved with their respective hosts and are strongly associated with one species or in some cases, such as Tula orthohantavirus (TULV), with several related species

---

* Corresponding author.
  E-mail address: rainer.ulrich@fli.de (R.G. Ulrich).
[1] Both authors contributed equally.

(Schmidt-Chanasit et al., 2010; Guterres et al., 2015; Milholland et al., 2018). However, cross-species transmission (host switch) is another important factor in hantavirus evolution (Ramsden et al., 2009; Guo et al., 2013; Bennett et al., 2014). Transmission to humans is usually mediated by inhalation of virus-contaminated aerosols such as feces and urine of infected hosts. Infections in humans can result in a subclinical course to severe illness, including hemorrhagic fever with renal syndrome (HFRS) with case fatality rate reaching 12% or hantavirus cardiopulmonary syndrome (HCPS) with case fatality rate up to 40% (Avšič-Županc et al., 2019).

The three genome segments are flanked by non-coding regions (NCR) that form panhandle-like structures (Spiropoulou, 2011). The S segment of 1530 to 2078 nucleotides (nt) encodes the nucleocapsid (N) protein of 428 to 433 amino acid (aa) residues (Plyusnin et al., 1994a). A nonstructural protein (NSs) is encoded in an overlapping open reading frame (ORF) in the S segment of orthohantaviruses carried by rodents of the family Cricetidae and might be important as an interferon inhibitor (Jääskelainen et al., 2007). The M segment is 3543–3801 nt long and encodes the glycoprotein precursor (GPC) that is cotranslationally cleaved into the amino-terminal Gn protein of 503–528 aa residues and the carboxy-terminal Gc protein of 479–486 aa residues (Sironen and Plyusnin, 2011). The L segment of 6529–6578 nt has the coding information for a 2147–2155 aa-residue long RNA-dependent RNA polymerase (RdRP) (Schmaljohn, 1990; Schlegel et al., 2014).

Voles of different *Myodes* and *Microtus* species represent hantavirus reservoirs. Whereas no hantavirus was detected in *Myodes* spp. in the New World so far (Milholland et al., 2018), several *Microtus* associated hantaviruses were found there. The reservoir of Prospect Hill orthohantavirus (PHV) has been identified as the meadow vole (*Microtus pennsylvanicus*) (Lee et al., 1985), but the virus has been also detected in montane vole (*Microtus montanus*) and prairie vole (*Microtus ochrogaster*) (Rowe et al., 1995). The montane vole was identified as the reservoir of El Moro Canyon orthohantavirus (Rowe et al., 1995). The California vole (*Microtus californicus*) represents the reservoir of Isla Vista virus, but was also found to be affected by spillover infection with Sin Nombre orthohantavirus, with the deer mouse (*Peromyscus maniculatus*) being the reservoir (Song et al., 1995; Turell et al., 1995).

In the Old World, *Myodes* spp. such as bank vole (*Myodes glareolus*), royal vole (also called Korean red-backed vole, *Myodes regulus*) and grey red-backed vole (*Myodes rufocanus*) transmit Puumala orthohantavirus (PUUV), which causes the majority of human hantavirus disease cases (Kariwa et al., 1995; Lundkvist et al., 1998; Song et al., 2007). TULV is one of the best studied and most broadly distributed orthohantaviruses. It is associated with the common vole (*Microtus arvalis*) and is genetically highly divergent with more than six phylogenetic clades, including the Eastern South (EST.S) clade with the Moravia prototype strain in the Czech Republic and the Central North (CEN.N) clade in the Northern part of Germany (Schmidt et al., 2016; Saxenhofer et al., 2019). TULV has been detected also in field voles (*Microtus agrestis*), narrow-headed voles (*Microtus gregalis*), East-European voles (*Microtus levis*), Altai voles (*Microtus obscurus*), European pine voles (*Microtus subterraneus*), and water voles (*Arvicola amphibius*) (Plyusnin et al., 1994b; Song et al., 2002; Scharninghausen et al., 2002; Plyusnina et al., 2008; Schmidt-Chanasit et al., 2010; Schlegel et al., 2012a; Polat et al., 2018). The field vole was recently identified as a reservoir of three closely-related hantaviruses: Traemmersee hantavirus (TRAV) in Germany (Jeske et al., 2019), Tatenale hantavirus (TATV) and Kielder hantavirus in Great Britain (Pounder et al., 2013; Thomason et al., 2017; Chappell et al., 2020). Additional hantaviruses, namely Khabarovsk orthohantavirus (KHAV), Fusong orthohantavirus, and Yuanjiang virus were found in Asia and are associated with Maximowicz's vole (*Microtus maximowiczii*) (Zou et al., 2008), reed vole (*Microtus fortis*) (Kariwa et al., 1995; Hörling et al., 1996; Zou et al., 2008) and root or tundra vole (*Microtus oeconomus*) (Plyusnina et al., 2008).

From the Baltic states in Europe, including Lithuania, Latvia and Estonia, only Dobrava-Belgrade orthohantavirus (DOBV) and PUUV

have been reported so far. Human infections with these viruses have been detected by serological methods (Lundkvist et al., 2002; Sandmann et al., 2005; Golovljova et al., 2007). Molecular evidence for the presence of these hantaviruses originated from the screening of bank voles in Lithuania and striped field mice (*Apodemus agrarius*) on the Estonian island Saaremaa (Nemirov et al., 1999; Straková et al., 2017). To date, nothing is known about the presence of hantaviruses in *Microtus* voles in this part of Europe. In this study, common voles, field voles, and root voles from multiple sites in Lithuania were screened by reverse transcription-polymerase chain reaction (RT-PCR) and immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) for the presence of TULV and related hantaviruses.

2. Material and methods

2.1. Collection of voles and dissection

Voles were trapped at 17 sites in Lithuania (Fig. 1; Table 1) during September, October and beginning of November 2015 to 2018, following methods described before (Balčiauskas, 2004). Morphological species identification was based on a dichotomous key previously described (Prūsaitė et al., 1988; Pucek, 1984). For selected animals, species identification was confirmed by dideoxy chain termination sequencing of cytochrome *b* PCR products and sequence comparison to GenBank entries as described elsewhere (Schlegel et al., 2012b). Weight, gender, and age class were determined for each carcass. Several tissue samples (lung, liver and kidney) were collected and stored at −20 °C. For detection of hantavirus-reactive antibodies tissue fluids were collected by thawing of previously frozen liver and lung tissue.

2.2. RNA isolation, RT-PCR, RNA ligation, conventional and high-throughput sequencing

RNA was extracted from lung tissue to screen for hantavirus infection using S segment specific RT-PCR as previously described (Schmidt et al., 2016). To determine complete genome sequences, total RNA of samples LT15/299, LT15/301, LT15/341 and LT15/351 was analyzed using hybrid sequence capture enrichment with subsequent high-throughput sequencing following Hiltbrunner and Heckel (2020). Consensus sequences of virus genomes were determined based on de novo assemblies of sequence reads called at a minimum base quality of $Q_{phred} = 33$ and 90% identity. Additional reference-based mapping of not assembled sequence reads was used to close short regions with low read coverage in some samples (see results; Saxenhofer et al., 2019; Hiltbrunner and Heckel, 2020). Furthermore, the complete coding sequences (CDS) of sample LT15/301 were determined by primer-walking (for primers see Table S1). Additionally, partial S, M and L segment sequences were generated for several RT-PCR positive samples by dideoxy chain termination sequencing of RT-PCR products including primer walking for selected samples (see Table S2). Sequences of the 5′ and 3′ NCRs of strain LT15/301 were determined by RT-PCR analysis of RNA molecules generated by RNA ligation using T4 RNA Ligase (Thermo Fisher, Waltham, MA, USA) following a published protocol (Klempa et al., 2006).

2.3. Phylogenetic analyses

Sequences were aligned using the Clustal W algorithm in Bioedit version 7.2.5. (Hall, 1999) (see Table S3). For nt sequences, the best-fitting substitution model was determined with jModelTest2 version 2.1.6, whereas on aa sequences ModelTest-NG version 0.1.5 was applied (Darriba et al., 2012; Darriba et al., 2020). The General Time Reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter was used in phylogenetic analyses with MrBayes version 3.2.7a with 1 to 3.5×10^7 generations and 25% burn-in and Maximum-Likelihood analyses with FastTreeMP version 2.1.10 with 1000 bootstraps. At the aa level, analyses with MrBayes used 8×10^6
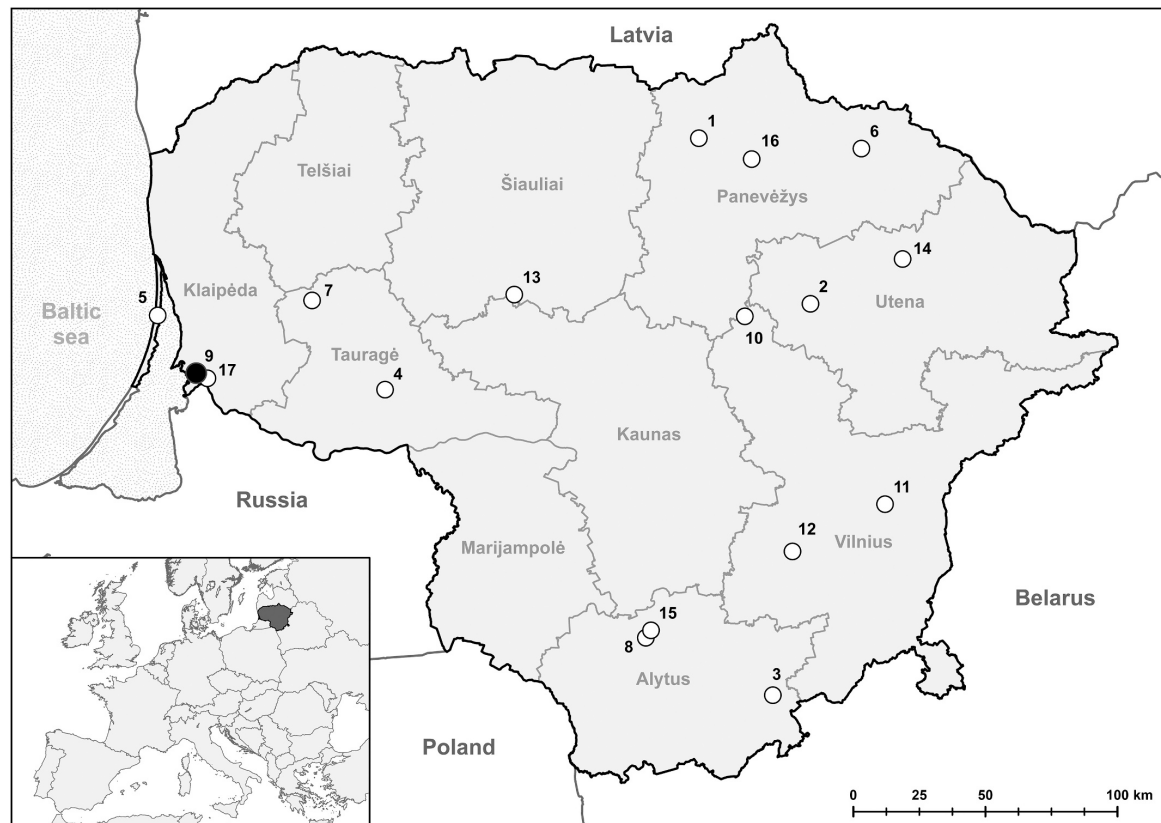
Fig. 1. Trapping sites of *Microtus* voles in Lithuania, Eastern Europe. Numbers correspond to trapping areas (see Table 1). Negative tested trapping sites are marked with white circles, and site Rusnė (9) with detection of Rusne virus is marked with a black circle.

generations, the retrovirus-specific (rtREV) and Whelan Goldman (WAG) substitution models and a burn-in phase of 25%. Maximum-Likelihood analyses were performed with FastTreeMP using the Jones-Taylor-Thornton (JTT) and the categories (CAT) model and 1000 bootstrap replicates. Consensus phylogenetic trees were drawn with 50% cut-off and posterior probability values greater than 95% and bootstrap values greater than 75 were reported at the nodes. All phylogenetic reconstructions were performed on CIPRES (Miller et al., 2010).

2.4. Pairwise evolutionary distance analysis

To test if the new Rusne virus and TRAV as well as TATV belong to the same virus species, pairwise evolutionary distance (PED) values were determined (Laenen et al., 2019). Available entire S and M segment CDS of hantaviruses were concatenated and translated to amino acid sequences. PED values were calculated using a maximum-likelihood approach with the WAG substitution model in Tree-Puzzle. Thottapalayam thottimvirus was used as an outgroup. A PED cut-off value of 0.1 was used for species demarcation within the family *Hantaviridae*.

2.5. Generation of recombinant N proteins and serological analysis

The complete N protein encoding sequence of strain LT17/R6 was amplified using primers RuNRES_FOR and RuNRES_REV (see Table S1)

and inserted into the *Xba*I-linearized pFX7-His plasmid (Ražanskienė et al., 2004). *Saccharomyces cerevisiae* strain Gcn2 was transformed with this plasmid, the synthesis of recombinant N protein of Rusne virus was initiated by adding galactose solution into YEPD medium. Recombinant N protein was purified by nickel chelate affinity chromatography as described before (Ražanskienė et al., 2004). The generation and purification of N proteins of TULV clade CEN.N, strain Thuringia, TULV clade EST.S, strain Moravia, and PUUV strains Vranica/Hällnäs and Bavaria have been described previously (Ražanskienė et al., 2004; Mertens et al., 2011; Jeske et al., 2019). Characterization of the Rusne virus antigen was done by ELISA and Western blot analysis using monoclonal antibodies (mAbs) produced against recombinant N proteins of PUUV, strain Vranica/Hällnäs and Sin Nombre/Andes orthohantaviruses (Kučinskaitė-Kodzė et al., 2011; Žvirblienė et al., 2006).

ELISA investigations of vole-derived chest cavity lavage (CCL) and tissue fluid samples with recombinant N proteins of Rusne virus, TULV strain Thuringia and PUUV strain Bavaria followed a standard protocol established for TULV (Schlegel et al., 2012a). Briefly, 0.2 μg/well of the recombinant protein were coated on 96-well polysorb Nunc-Immuno plates (VWR International GmbH, Hannover, Germany) and incubated for 1 h with tissue fluid or CCL diluted 1:10. After washing, a goat anti-mouse IgG (H + L) labeled with horse-radish peroxidase (BioRad, Munich, Germany) was used to detect antibodies against the specific hantavirus antigen. Finally, 100 μl of tetramethylbenzidine (TMB) peroxidase EIA substrate (BioRad, Munich, Germany) was added and

3

60

Table 1
Results of RT-PCR and IgG ELISA hantavirus screening of voles collected in Lithuania during 2015–2018.

Site (number in Fig. 1)	Year	Species	Results (number of positive/total number of investigated voles)	
			RT-PCR	IgG ELISA
Aukštikalniai (1)	2018	*Microtus arvalis*	0/1	0/1
Ažuožeriai (2)	2018	*Microtus arvalis*	0/30	0/30
		Microtus oeconomus	0/4	0/4
Barčiai (3)	2018	*Microtus arvalis*	0/11	0/11
Gaurė (4)	2018	*Microtus arvalis*	0/4	0/4
		Microtus oeconomus	0/2	0/2
Juodkrantė (5)	2015	*Microtus agrestis*	0/1	0/1
		Microtus oeconomus	0/2	0/2
Kalpokai (6)	2018	*Microtus arvalis*	0/7	0/7
Kvėdarna (7)	2018	*Microtus agrestis*	0/1	0/1
Luksnėnai (8)	2018	*Microtus arvalis*	0/6	0/6
		Microtus oeconomus	0/3	0/3
Rusnė (9)	2015	*Microtus agrestis*	0/12	0/12
		Microtus oeconomus	**6/42**	**4/42**
	2016	*Microtus agrestis*	0/7	0/7
		Microtus oeconomus	**1/7**	0/7
	2017	*Microtus agrestis*	0/4	0/4
		Microtus arvalis	0/1	0/1
		Microtus oeconomus	**1/8**	0/8
	2018	*Microtus agrestis*	0/10	0/10
		Microtus oeconomus	0/3	0/3
Taujėnai (10)	2018	*Microtus arvalis*	0/9	0/9
		Microtus oeconomus	0/1	0/1
Tauragirė (11)	2017	*Microtus oeconomus*	0/1	0/1
Trakai (12)	2016	*Microtus arvalis*	0/11	0/11
Tytuvėnai (13)	2018	*Microtus agrestis*	0/1	0/1
		Microtus arvalis	0/1	0/1
		Microtus oeconomus	0/1	0/1
Užpaliai (14)	2018	*Microtus arvalis*	0/9	0/9
Užubaliai (15)	2018	*Microtus arvalis*	0/4	0/4
Vabalninkas (16)	2017	*Microtus arvalis*	0/15	0/15
		Microtus oeconomus	0/1	0/1
Žalgiriai (17)	2017	*Microtus agrestis*	0/10	0/10
		Microtus oeconomus	0/4	0/4
Total		*Microtus agrestis*	0/46	0/46
		Microtus arvalis	0/109	0/109
		Microtus oeconomus	**8/79**	**4/79**

Rusne hantavirus RNA and/or anti-Rusne hantavirus antibody positive root voles are given in bold.

incubated for 10 min in the dark. The reaction was stopped by addition of 100 μl 1 M sulfuric acid. Subsequently, optical density was measured with Plate reader Infinite 200 PRO (Tecan, Männedorf, Switzerland) at 450 nm (reference at 620 nm). The mAb 5E11 (Kučinskaitė-Kodzė et al., 2011) was used as positive control. Bank vole (PUUV) and common vole (TULV) CCL samples from previous studies (Drewes et al., 2017; Jeske et al., submitted) or root vole tissue fluid tested negative by the respective hantavirus-IgG ELISA and RT-PCR were applied as negative controls. Lower and upper cut-off values were determined according to a previous study (Mertens et al., 2011). For the cross-reactivity study three anti-PUUV-positive bank vole and three anti-TULV-positive common vole CCL samples were used originating from previous investigations (Drewes et al., 2017; Jeske et al., submitted).

3. Results

3.1. RT-PCR screening of voles

A total of 234 voles including 79 root voles, 46 field voles and 109 common voles were collected during small mammal trapping sessions at 17 sites in Lithuania during 2015–2018 (Fig. 1). Hantavirus RT-PCR screening resulted in the identification of eight positive samples (Table 1). All positive samples were root voles collected in 2015, 2016 and 2017 at Rusnė site. The eight hantavirus RNA positive samples

originated from five adult and one juvenile male, and one adult and one subadult female (Table S2). Sympatrically occurring field voles (N = 33) and a single common vole from this site were hantavirus RNA negative. None of the voles from any of the other trapping sites were found to be hantavirus RNA positive.

RT-PCR-mediated generation of partial S and L segment sequences indicated a novel hantavirus strain in the root voles, designated according to the trapping site "Rusne virus". The strain LT15/301 has the highest nucleotide and amino acid sequence similarity to the other Rusne virus strains (0.987–1.0/1.0) and then to the recently discovered Traemmersee hantavirus (TRAV; 0.809–0.837/0.963–0.977) and Tatenale hantavirus (TATV; 0.796–0.834/0.954–0.99) (see Fig. S2, Table S4). The L segment/RdRP sequences of British Kielder hantavirus strains showed similarities of 0.816–0.819/0.981–0.99 to the Rusne virus strains (Fig. S2, Table S4).

3.2. Complete genome sequence determination and phylogenetic analysis

To generate a complete genome sequence of this novel hantavirus, a hybrid sequence capture approach was followed for samples LT15/299, LT15/301, LT15/341 and LT15/351. This allowed us to obtain genome sequences of the Rusne virus with mean sequence read depths of the assemblies of 422x (LT15/299), 51x (LT15/301), 30x (LT15/341) and 61x (LT15/351). For three samples, the gapless assemblies covered the complete coding sequences (CDS) of S segment, M segment and L segment, and at least parts of the 5′ and 3′ NCRs (Tables S2 and S6). The NGS-derived L segment sequence of LT15/341 lacked 199 nt and 4% of the nt were called at a read depth less than 5x compared to 0%, 0.3% and 0.1% in the other assemblies. However, the determined parts of L segment and the entire CDS of S and M segments were identical to those of LT15/351. In parallel, a primer walking based approach resulted in the generation of complete CDS of S, M and L segments of strain LT15/301 (Table S2). The NGS-derived and primer-walking approach-based sequences of LT15/301 were identical except for one nucleotide difference over the whole L CDS and one different nucleotide in the M CDS. Sequence read coverage of the NGS assembly was at least 30x at these positions and there were no sequence reads with the nucleotide determined in the primer-walking-based sequence.

The S segment had a total length of 2059 nt and encodes a N protein of 433 aa residues (Table S6). The putative NSs protein of Rusne virus strains LT15/301 and LT17/R6 had an amino-terminal extension of four residues, similar to the extension of five residues in the corresponding putative protein of KHAV, but different from that of TRAV and TATV and other vole-borne hantaviruses. The S segment 5′ and 3′ NCR sequences of strain LT15/301 were identified to be of 42 nt and 715 nt, respectively (Table S6). The 5′ NCR sequences were highly conserved in length before the start codon and for the first 30 nt in particular (Fig. 2), whereas the 3′ NCR sequence showed a higher sequence variability with two 26–53 nt long insertions/deletions, but a more conserved sequence at the terminal 100 nt (data not shown).

The M segment was predicted to encode a GPC of 1148 aa residues and contained the conserved WAASA cleavage motif between Gn and Gc at aa residues 654–658; the NGS allowed the determination of 28 and 214 nt of the NCR sequences (Tables S2 and S6). The L segment had a coding sequence of 6465 nt and codes for the RdRP of 2155 aa residues. Again, only partial sequences at both NCR ends were determined by NGS (Tables S2 and S6).

The S, M and L segment CDS as well as the N, GPC and RdRP amino acid sequences showed the highest similarity to TRAV and TATV sequences, with overall pairwise CDS nt and aa sequence similarity ranging between 0.802 and 0.847 and 0.939–0.976, respectively (Table S5). The nt and aa sequences of the three viruses formed a monophyletic group in all phylogenetic analyses (Fig. 3A–F).

Comparison of concatenated N and GPC aa sequences of Rusne virus resulted in PED values below 0.1 to TRAV and TATV (Table S5). This indicates that the novel Rusne virus discovered in *M. oeconomus* and
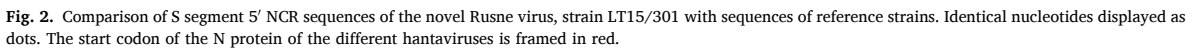
Fig. 2. Comparison of S segment 5′ NCR sequences of the novel Rusne virus, strain LT15/301 with sequences of reference strains. Identical nucleotides displayed as dots. The start codon of the N protein of the different hantaviruses is framed in red.

Table 2
Reactivity of recombinant nucleocapsid (N) proteins of orthohantaviruses with monoclonal antibodies (mAbs) in ELISA and Western blot test.

		Reactivity of mAbs in ELISA/Western blot test					
		4H3	7G2	2C6	5C5	5E11	7A5
Recombinant N proteins	Rusne virus	−/−	+/+	−/−	+/+	+/+	+/+
	TULV-Moravia	−/−	+/+	−/−	+/+	+/+	(+)/+
	PUUV-Bawa	−/−	+/+	−/−	+/+	+/+	+/+
	PUUV-Vra	n.d./n.d.	+/n.d.	+/+	+/n.d.	+/n.d.	+/n.d.
	SNV	+	+/+	−/−	+/+	+/+	+/+

n.d., not determined; +, positive; (+), weakly positive; −, negative.
TULV-Moravia, Tula virus, strain Moravia; PUUV-Bawa, Puumala virus, strain Bavaria; PUUV-Vra, Puumala virus, strain Vranica/Hällnäs; SNV, Sin Nombre virus.

TRAV and TATV detected in *M. agrestis* belong to the same tentative orthohantavirus species.

3.3. Cross-reactivity of recombinant N protein of Rusne virus and serological screening of voles

The entire N protein of Rusne virus was produced in *S. cerevisiae* yeast and purified by affinity chromatography. The N protein of Rusne virus was tested in parallel with N antigens of TULV strain Moravia, PUUV strains Bavaria and Vranica/Hällnäs, and SNV for cross-reactivity. A high cross-reactivity was documented by ELISA and Western blot analyses using PUUV-specific mAbs 5C5, 5E11 and 7A5 and SNV/ANDV-specific mAb 7G2 (Table 2). In addition, three of three anti-TULV-positive common vole CCL samples and two of three anti-PUUV-positive bank vole CCL samples reacted in the ELISA with Rusne virus antigen (data not shown).

ELISA screening of tissue fluids of all 234 voles resulted in the detection of four seropositive root voles (Table 1 and Table S2). All four ELISA-positive samples originated from Rusne virus-RNA positive voles; four additional RNA-positive samples were negative in this novel ELISA (Table S2). The IgG ELISA positive samples consisted of two males and two females, from which one female was subadult and the other three voles were adult individuals.

4. Discussion

We detected a *Microtus*-associated hantavirus in Lithuania, Eastern Europe. The multiple detection of this novel virus in root voles at one site over time suggests this vole species as the reservoir of this novel hantavirus. This conclusion was further strengthened by the absence of Rusne virus RNA in the field voles collected at the same trapping site.

We conclude that the new Rusne virus is a strain of TRAV recently detected in Germany and TATV discovered in Great Britain, due to their sequence similarity with a PED value below the definition level of a new species as stated by the International Committee on Taxonomy of Viruses (ICTV) (Pounder et al., 2013; Thomason et al., 2017; Jeske et al., 2019; Chappell et al., 2020; ICTV, 2020). On the basis of partial L segment sequences Rusne virus, TRAV and TATV are also very similar to British Kielder hantavirus. It appears that they all belong to one tentative orthohantavirus species, given high levels of genetic divergence also within other European orthohantavirus species and the importance of geographic isolation for evolutionary processes in hantaviruses (Weber de Melo et al., 2015; Saxenhofer et al., 2017; Saxenhofer et al., 2019; Hiltbrunner and Heckel, 2020).

Interestingly, TRAV in Germany as well as Tatenale and Kielder hantaviruses in Great Britain were only detected in field voles (Pounder et al., 2013; Thomason et al., 2017; Jeske et al., 2019). In this study, Rusne virus was exclusively detected in root voles, but not in sympatric field voles. The root vole is the only member of the genus *Microtus* with a holarctic distribution from the German-Polish border through Asia, Alaska and Canada, including isolated populations in some regions of Europe, e.g. in the Netherlands (von Tast, 1982; Brunhoff et al., 2003; Fink et al., 2010). The root vole invaded Lithuania relatively recently, with first findings in 1964 from the south and south-western parts and subsequently colonized most of Lithuania (Ivanauskas et al., 1964; Balčiauskas et al., 2010). In the Nemunas River Delta with the Rusnė trapping site, the root vole joins the striped field mouse (*Apodemus agrarius*) as dominant small mammals, and is found in higher proportions than in other regions of Lithuania (Balčiauskas et al., 2012). Interestingly, one root vole was previously found to harbor Fusong orthohantavirus (FUSV), strain Vladivostok, in Russia (Plyusnina et al., 2008). However, the reservoir host of FUSV is the reed vole and the detection of FUSV in the single root vole might represent a spillover infection (Kariwa et al., 1999; Plyusnina et al., 2008; Zou et al., 2008).

At present, it is unclear if the detection of Rusne virus in root voles in Lithuania, TRAV in a field vole in Germany and TATV and Kielder hantavirus in field voles in Britain might be the result of a host switch of an ancestral virus in the past. Both vole species are present in Eastern Germany, as well as Eastern Europe in general with similar habitats, but root voles are absent from the British Isles (Kryštufek et al., 2007; Linzey
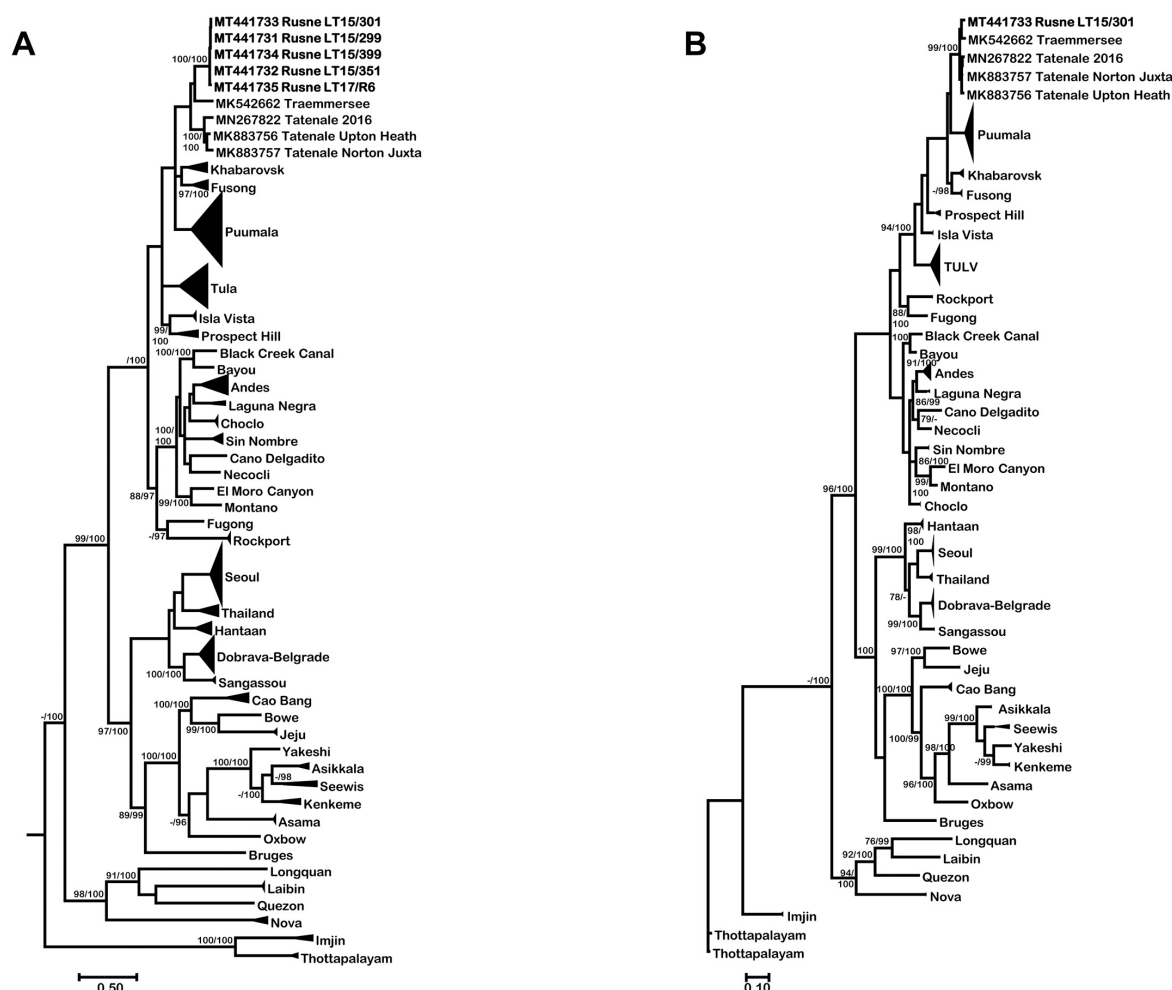
5

Fig. 3. Consensus phylogenetic trees of complete coding sequences (CDS) of S segment (A), M segment (C) and L segment CDS (E) and amino acid sequences of complete nucleocapsid protein (B), glycoprotein precursor protein (D), and RNA-dependent RNA polymerase (F). Alignments were constructed using the Clustal W Multiple Alignment algorithm implemented in Bioedit V7.2.3. (Hall, 1999). The consensus nt sequence trees are based on Bayesian analyses with up to 3.5×10^7 generations and a burn-in phase of 25%, and Maximum-Likelihood analyses with 1000 bootstraps and 50% cut-off using the General Time Reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter for both algorithms. Phylogenetic consensus trees of the complete amino acid sequences of the nucleocapsid (B), complete glycoprotein precursor protein (D), and the complete RNA-dependent RNA polymerase (F) were constructed with Bayesian algorithms with 8×10^6 generations using the retrovirus-specific (rtREV) and Whelan Goldman (WAG) substitution models and with Maximum Likelihood algorithms, the Jones-Taylor-Thornton (JTT) and CAT substitution models and 1000 bootstrap replicates. Bootstrap values were only transferred to the Bayesian trees, if branches were consistent. Posterior probability values >95%/bootstrap values >70 are given at the supported nodes. The tree reconstructions were done on CIPRES (Miller et al., 2010). Names in bold indicate newly generated sequences (GenBank accession numbers MT441731 – MT441741). Triangles indicate condensed branches; for all hantavirus sequences included see Table S3.

et al., 2016). To determine if host switch has taken place and what was the original host of Rusne virus, TRAV and TATV further studies in field voles, root voles and other *Microtus* voles in Eurasia are needed.

Rusne virus RNA was more frequently detected than Rusne virus-reactive antibodies. As we used here the new Rusne virus N protein - a homologous antigen - in the ELISA, this discrepancy cannot be explained by the use of a heterologous antigen. One explanation might be the use of highly diluted tissue fluids. Alternatively, this discrepancy might be caused by seronegative root voles being in the acute phase of the infection. Therefore, a screening of voles for hantavirus infection may

profit from a molecular approach instead of a serologic assay (Weber de Melo et al., 2015), in particular for selection of samples for virus isolation (Binder et al., 2020).

The high aa sequence similarity between N proteins of Rusne virus, PUUV and TULV was reflected in a strong cross-reactivity of these antigens as evidenced by ELISA and Western blot investigations exploiting mAbs and polyclonal sera. Therefore, the detection of PUUV- or TULV-reactive antibodies in human serum samples, as e.g. in Lithuania (Sandmann et al., 2005; Dargevičius et al., 2007), might be misinterpreted in regions where TRAV/Rusne virus/TATV/Kielder virus
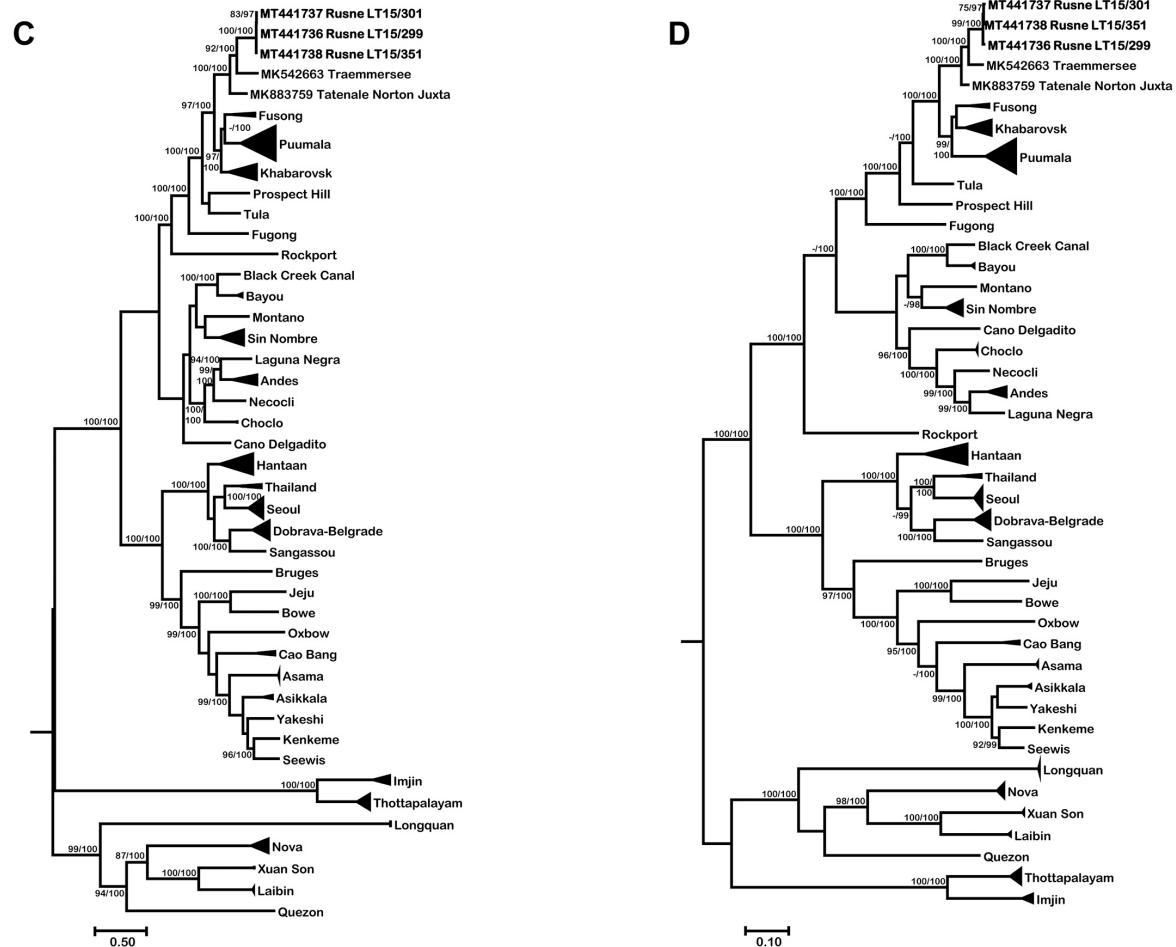
Fig. 3. (*continued*).

circulates. For a definite proof of potential human infections with this virus species, a virus isolate for focus reduction neutralization test use is urgently needed (Krüger et al., 2001).

5. Conclusions

In this study we detected the first root vole-associated hantavirus in Europe, Rusne virus, that forms a putative hantavirus species together with TRAV and TATV. The multiple detection of similar sequences of this novel virus in a root vole population during three years suggests this vole species as the reservoir host. We further developed a Rusne virus antigen that might be used in serological screenings of human serum samples. A Eurasian wide screening of root voles, field voles and other *Microtus* voles is needed to evaluate the geographic range and possible host association of Rusne virus, TRAV and TATV strains. In the future, isolation of strains of these viruses is needed for the development of additional serological detection tests of human infections including neutralization assays.

Author contributions

Stephan Drewes: Conceptualization, Visualization, Writing - original draft, Investigation, Writing - review & editing. Kathrin Jeske: Conceptualization, Visualization, Writing - original draft, Investigation, Writing - review & editing. Petra Straková: Investigation, Writing - review & editing. Linas Balčiauskas: Investigation, Data curation, Writing - review & editing. René Ryll: Investigation, Formal analysis, Writing - review & editing. Laima Balčiauskienė: Investigation, Data curation, Writing - review & editing. David Kohlhause: Investigation. Guy-Alain Schnidrig: Investigation, Formal analysis. Melanie Hiltbrunner: Investigation, Data curation. Aliona Špakova: Investigation, Data curation. Rasa Insodaitė: Investigation, Formal analysis. Rasa Petraitytė-Burneikienė: Supervision, Writing - review & editing. Gerald Heckel: Conceptualization, Writing - original draft, Supervision, Writing - review & editing, Project administration, Funding acquisition. Rainer G. Ulrich: Conceptualization, Writing - original draft, Supervision, Writing - review & editing, Project administration.

Ethical statement

Rodent sampling was conducted with permission from the Environmental Protection Agency (EPA) and approved by the Ministry of Environment of the Republic of Lithuania, licenses No. 22 (2015-04-10), No. 12 (2016-03-30), No 13 (2017-03-22) and No 6 (2018-02-02) in
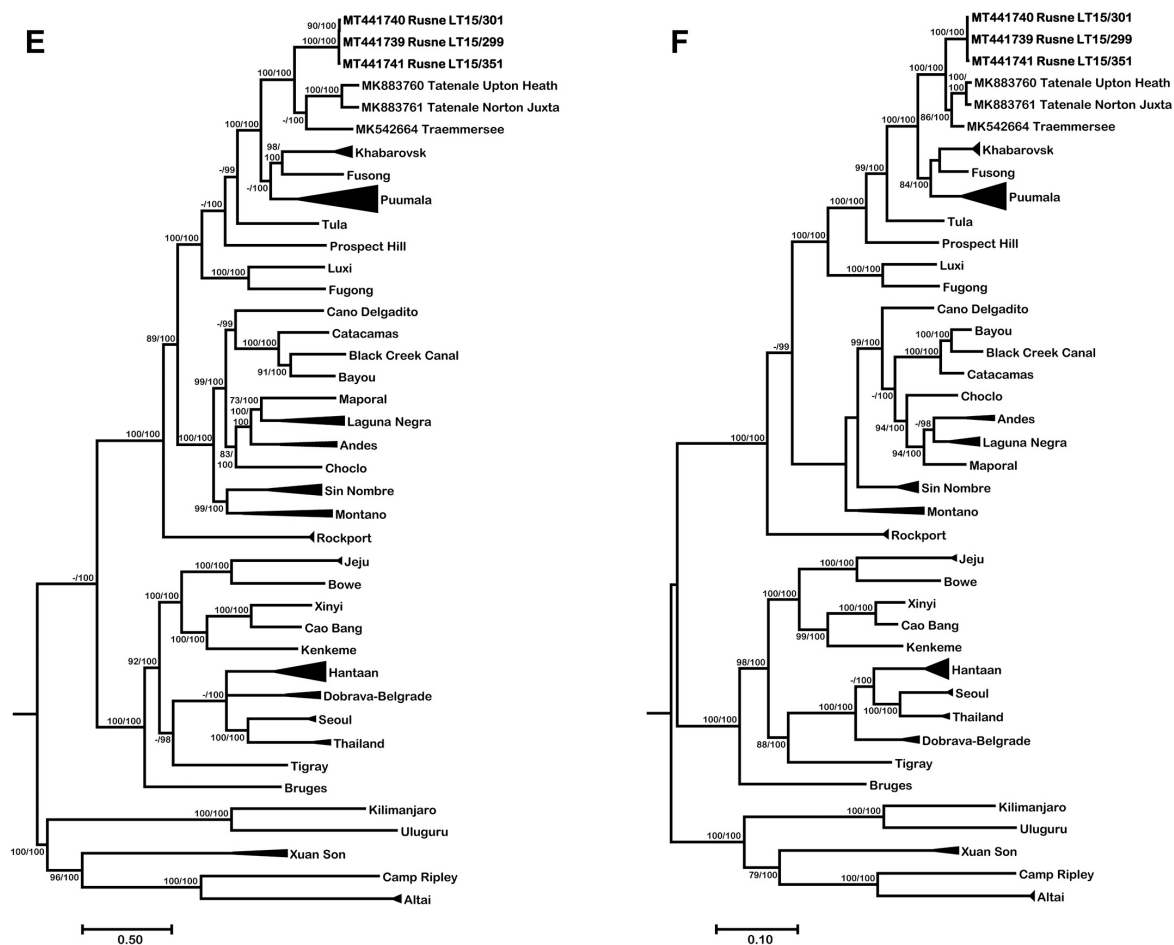
7

Fig. 3. (*continued*).

accordance with Lithuanian (the Republic of Lithuania Law on Welfare and Protection of Animals No. XI-2271) and European legislation (Directive 2010/63/EU) on the protection of animals.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2020.104520.

References

Avšič-Županc, T., Saksida, A., Korva, M., 2019. Hantavirus infections. Clin. Microbiol. Infect. e6–e16. https://doi.org/10.1111/1469-0691.12291.

Balčiauskas, L., 2004. Methods of Investigation of Terrestrial Ecosystems. Part I. Animal Surveys. VUL, Vilnius, Lithuania (in Lithuanian).

Balčiauskas, L., Balčiauskienė, L., Baltrūnaitė, L., 2010. Root vole, *Microtus oeconomus*, in Lithuania: changes in the distribution range. Folia Zool. 59, 267–278. https://doi.org/10.25225/fozo.v59.i4.a1.2010.

Balčiauskas, L., Balčiauskienė, L., Janonytė, A., 2012. The influence of spring floods on small mammal communities in the Nemunas River Delta, Lithuania. Biologia 67, 1220–1229. https://doi.org/10.2478/s11756-012-0116-8.

Bennett, S.N., Gu, S.H., Kang, H.J., Arai, S., Yanagihara, R., 2014. Reconstructing the evolutionary origins and phylogeography of hantaviruses. Trends Microbiol. 22, 473–482. https://doi.org/10.1016/j.tim.2014.04.008.

Binder, F., Reiche, S., Roman-Sosa, G., Saathoff, M., Ryll, R., Trimpert, J., Kunec, D., Höper, D., Ulrich, R.G., 2020. Isolation and characterization of new Puumala orthohantavirus strains from Germany. Virus Genes 56, 448–460. https://doi.org/10.1007/s11262-020-01755-3.

Brunhoff, C., Galbreath, K.E., Fedorov, V.B., Cook, J.A., Jaarola, M., 2003. Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late quaternary biogeography of high latitudes. Mol. Ecol. 12, 957–968. https://doi.org/10.1046/j.1365-294x.2003.01796.x.

8

Chappell, J.G., Tsoleridis, T., Onianwa, O., Drake, G., Ashpole, I., Dobbs, P., Edema, W., Kumi-Ansah, F., Bennett, M., Tarlinton, R.E., Ball, J.K., McClure, C.P., 2020. Retrieval of the complete coding sequence of the UK-endemic Tatenale orthohantavirus reveals extensive strain variation and supports its classification as a novel species. Viruses 12. https://doi.org/10.3390/v12040454.

Dargevičius, A., Petraitytė, R., Sribikienė, B., Sileikienė, E., Razukevičienė, L., Žiginskienė, E., Vorobjovienė, R., Ražanskienė, A., Sasnauskas, K., Bumblytė, I.A., Kuzminskis, V., 2007. Prevalence of antibodies to hantavirus among hemodialysis patients with end-stage renal failure in Kaunas and its district. Medicina 43 (Suppl. 1), 72–76.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772. https://doi.org/10.1038/nmeth.2109.

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., Flouri, T., 2020. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. Mol. Biol. Evol. 37, 291–294. https://doi.org/10.1093/molbev/msz189.

Drewes, S., Ali, H.S., Saxenhofer, M., Rosenfeld, U.M., Binder, F., Cuypers, F., Schlegel, M., Röhrs, S., Heckel, G., Ulrich, R.G., 2017. Host-associated absence of human Puumala virus infections in Northern and Eastern Germany. Emerg. Infect. Dis. 23, 83–86. https://doi.org/10.3201/eid2301.160224.

Fink, S., Fischer, M.C., Excoffier, L., Heckel, G., 2010. Genomic scans support repetitive continental colonization events during the rapid radiation of voles (Rodentia: *Microtus*): the utility of AFLPs versus mitochondrial and nuclear sequence markers. Syst. Biol. 59, 548–572. https://doi.org/10.1093/sysbio/syq042.

Golovljova, I., Vasilenko, V., Mittzenkov, V., Prukk, T., Seppet, E., Vene, S., Settergren, B., Plyusnin, A., Lundkvist, Å., 2007. Characterization of hemorrhagic fever with renal syndrome caused by hantaviruses, Estonia. Emerg. Infect. Dis. 13, 1773–1776. https://doi.org/10.3201/eid1311.070221.

Guo, W.P., Lin, X.D., Wang, W., Tian, J.H., Cong, M.L., Zhang, H.L., Wang, M.R., Zhou, R. H., Wang, J.B., Li, M.H., Xu, J., Holmes, E.C., Zhang, Y.Z., 2013. Phylogeny and origins of hantaviruses harbored by bats, insectivores, and rodents. PLoS Pathog. 9, e1003159 https://doi.org/10.1371/journal.ppat.1003159.

Guterres, A., de Oliveira, R.C., Fernandes, J., de Lemos, E.R., 2015. Is the evolution of hantavirus driven by its host? Infect. Genet. Evol. 35, 142–143. https://doi.org/10.1016/j.meegid.2015.08.002.

Hall, T.A., 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. In: Nucleic Acids Symp. Ser, 41. Information Retrieval Ltd., pp. 95–98 (c1979-c2000).

Hiltbrunner, M., Heckel, G., 2020. Assessing genome-wide diversity in European hantaviruses through sequence capture from natural host samples. Viruses 12, E749. https://doi.org/10.3390/v12070749.

Hörling, J., Chizhikov, V., Lundkvist, Å., Jonsson, M., Ivanov, L., Dekonenko, A., Niklasson, B., Dzagurova, T., Peters, C.J., Tkachenko, E., Nichol, S., 1996. Khabarovsk virus: a phylogenetically and serologically distinct hantavirus isolated from *Microtus fortis* trapped in far-East Russia. J. Gen. Virol. 77 (Pt 4), 687–694. https://doi.org/10.1099/0022-1317-77-4-687.

ICTV, 2020. International Committee on Taxonomy of Viruses. https://talk.ictvonline.org/taxonomy/ (accessed 16 February 2020).

Ivanauskas, T., Likevičienė, N., Maldžiūnaitė, S., 1964. Guide-Book of Lithuanian Mammals. Valstybinė Politinės ir Mokslinės Literatūros Leidykla, Vilnius (in Lithuanian).

Jääskelainen, K.M., Kaukinen, P., Minskaya, E.S., Plyusnina, A., Vapalahti, O., Elliott, R. M., Weber, F., Vaheri, A., Plyusnin, A., 2007. Tula and Puumala hantavirus NSs ORFs are functional and the products inhibit activation of the interferon-beta promoter. J. Med. Virol. 79, 1527–1536. https://doi.org/10.1002/jmv.20948.

Jeske, K., Hiltbrunner, M., Drewes, S., Ryll, R., Wenk, M., Špakova, A., Petraitytė-Burneikienė, R., Heckel, G., Ulrich, R.G., 2019. Field vole-associated Traemmersee hantavirus from Germany represents a novel hantavirus species. Virus Genes 55, 848–853. https://doi.org/10.1007/s11262-019-01706-7.

Kariwa, H., Yoshizumi, S., Arikawa, J., Yoshimatsu, K., Takahashi, K., Takashima, I., Hashimoto, N., 1995. Evidence for the existence of Puumula-related virus among *Clethrionomys rufocanus* in Hokkaido, Japan. Am. J. Trop. Med. Hyg. 53, 222–227. https://doi.org/10.4269/ajtmh.1995.53.222.

Kariwa, H., Yoshimatsu, K., Sawabe, J., Yokota, E., Arikawa, J., Takashima, I., Fukushima, H., Lundkvist, Å., Shubin, F.N., Isachkova, L.M., Slonova, R.A., Leonova, G.N., Hashimoto, N., 1999. Genetic diversities of hantaviruses among rodents in Hokkaido, Japan and far East Russia. Virus Res. 59, 219–228. https://doi.org/10.1016/s0168-1702(98)00141-5.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Denys, C., Koivogui, L., ter Meulen, J., Krüger, D.H., 2006. Hantavirus in African wood mouse, Guinea. Emerg. Infect. Dis. 12, 838–840. https://doi.org/10.3201/eid1205.051487.

Krüger, D.H., Ulrich, R., Lundkvist, Å., 2001. Hantavirus infections and their prevention. Microbes Infect. 3, 1129–1144. https://doi.org/10.1016/s1286-4579(01)01474-5.

Kryštufek, B., Vohralík, V., Zima, J., Zagorodnyuk, I., 2007. *Microtus agrestis*, the IUCN Red List of Threatened Species 2007: e.T13426A3928014. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T13426A22349665.en.

Kučinskaitė-Kodzė, I., Petraitytė-Burneikienė, R., Žvirblienė, A., Hjelle, B., Medina, R.A., Gedvilaitė, A., Ražanskienė, A., Schmidt-Chanasit, J., Mertens, M., Padula, P., Sasnauskas, K., Ulrich, R.G., 2011. Characterization of monoclonal antibodies against hantavirus nucleocapsid protein and their use for immunohistochemistry on rodent and human samples. Arch. Virol. 156, 443–456. https://doi.org/10.1007/s00705-010-0879-6.

Laenen, L., Vergote, V., Calisher, C.H., Klempa, B., Klingström, J., Kuhn, J.H., Maes, P., 2019. Hantaviridae: current classification and future perspectives. Viruses 11. https://doi.org/10.3390/v11090788.

Lee, P.W., Amyx, H.L., Yanagihara, R., Gajdusek, D.C., Goldgaber, D., Gibbs Jr., C.J., 1985. Partial characterization of Prospect Hill virus isolated from meadow voles in the United States. J. Infect. Dis. 152, 826–829. https://doi.org/10.1093/infdis/152.4.826.

Linzey, A.V., Shar, S., Lkhagvasuren, D., Juškaitis, R., Sheftel, B., Meinig, H., Amori, G., Henttonen, H., 2016. *Microtus oeconomus* (Errata version published in 2017). The IUCN Red List of Threatened Species 2016: e.T13451A115113894. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T13451A22347188.en.

Lundkvist, Å., Wiger, D., Hörling, J., Sjolander, K.B., Plyusnina, A., Mehl, R., Vaheri, A., Plyusnin, A., 1998. Isolation and characterization of Puumala hantavirus from Norway: evidence for a distinct phylogenetic sublineage. J. Gen. Virol. 79 (Pt 11), 2603–2614. https://doi.org/10.1099/0022-1317-79-11-2603.

Lundkvist, Å., Lindegren, G., Brus Sjolander, K., Mavtchoutko, V., Vene, S., Plyusnin, A., Kalnina, V., 2002. Hantavirus infections in Latvia. Eur. J. Clin. Microbiol. Infect. Dis. 21, 626–629. https://doi.org/10.1007/s10096-002-0784-3.

Mertens, M., Hofmann, J., Petraitytė-Burneikienė, R., Ziller, M., Sasnauskas, K., Friedrich, R., Niederstrasser, O., Krüger, D.H., Groschup, M.H., Petri, E., Werdermann, S., Ulrich, R.G., 2011. Seroprevalence study in forestry workers of a non-endemic region in eastern Germany reveals infections by Tula and Dobrava-Belgrade hantaviruses. Med. Microbiol. Immunol. 200, 263–268. https://doi.org/10.1007/s00430-011-0203-4.

Milholland, M.T., Castro-Arellano, I., Suzán, G., Garcia-Peña, G.E., Lee Jr., T.E., Rohde, R.E., Alonso Aguirre, A., Mills, J.N., 2018. Global diversity and distribution of hantaviruses and their hosts. EcoHealth 15, 163–208. https://doi.org/10.1007/s10393-017-1305-2.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees, 2010 Gateway Computing Environments Workshop (GCE). IEEE, pp. 1–8.

Nemirov, K., Vapalahti, O., Lundkvist, Å., Vasilenko, V., Golovljova, I., Plyusnina, A., Niemimaa, J., Laakkonen, J., Henttonen, H., Vaheri, A., Plyusnin, A., 1999. Isolation and characterization of Dobrava hantavirus carried by the striped field mouse (*Apodemus agrarius*) in Estonia. J. Gen. Virol. 80 (Pt 2), 371–379. https://doi.org/10.1099/0022-1317-80-2-371.

Plyusnin, A., Vapalahti, O., Ulfves, K., Lehväslaiho, H., Apekina, N., Gavrilovskaya, I., Blinov, V., Vaheri, A., 1994a. Sequences of wild Puumala virus genes show a correlation of genetic variation with geographic origin of the strains. J. Gen. Virol. 75 (Pt 2), 405–409. https://doi.org/10.1099/0022-1317-75-2-405.

Plyusnin, A., Vapalahti, O., Lankinen, H., Lehväslaiho, H., Apekina, N., Myasnikov, Y., Kallio-Kokko, H., Henttonen, H., Lundkvist, Å., Brummer-Korvenkontio, M., Gavrilovskaya, I., Vaheri, A., 1994b. Tula virus: a newly detected hantavirus carried by European common voles. J. Virol. 68, 7833–7839. https://doi.org/10.1128/jvi.68.12.7833-7839.1994.

Plyusnina, A., Laakkonen, J., Niemimaa, J., Nemirov, K., Muruyeva, G., Pohodiev, B., Lundkvist, Å., Vaheri, A., Henttonen, H., Vapalahti, O., Plyusnin, A., 2008. Genetic analysis of hantaviruses carried by *Myodes* and *Microtus* rodents in Buryatia. Virol. J. 5, 4. https://doi.org/10.1186/1743-422x-5-4.

Polat, C., Ergunay, K., Irmak, S., Erdin, M., Brinkmann, A., Cetintas, O., Cogal, M., Sozen, M., Matur, F., Nitsche, A., Oktem, I.M.A., 2018. A novel genetic lineage of Tula orthohantavirus in Altai voles (*Microtus obscurus*) from Turkey. Infect. Genet. Evol. 67, 150–158. https://doi.org/10.1016/j.meegid.2018.11.015.

Pounder, K.C., Begon, M., Sironen, T., Henttonen, H., Watts, P.C., Voutilainen, L., Vapalahti, O., Klempa, B., Fooks, A.R., McElhinney, L.M., 2013. Novel hantavirus in wildlife, United Kingdom. Emerg. Infect. Dis. 19, 673–675. https://doi.org/10.3201/eid1904.121057.

Prūsaitė, J., Mažeikytė, R., Pauža, D., Paužienė, N., Baleišis, R., Juškaitis, R., Mickus, A., Grušas, A., Skeiveris, R., Bluzma, P.M., 1988. Fauna of Lithuania. Mammals, Vilnius (in Lithuanian).

Pucek, Z., 1984. Key for Identification of Polish Mammals. PWN – Polish Scientific Publishers, Warsaw (in Polish).

Ramsden, C., Holmes, E.C., Charleston, M.A., 2009. Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for codivergence. Mol. Biol. Evol. 26, 143–153. https://doi.org/10.1093/molbev/msn234.

Ražanskienė, A., Schmidt, J., Geldmacher, A., Ritzi, A., Niedrig, M., Lundkvist, Å., Krüger, D.H., Meisel, H., Sasnauskas, K., Ulrich, R., 2004. High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast *Saccharomyces cerevisiae*. J. Biotechnol. 111, 319–333. https://doi.org/10.1016/j.jbiotec.2004.04.010.

Rowe, J.E., St Jeor, S.C., Riolo, J., Otteson, E.W., Monroe, M.C., Henderson, W.W., Ksiazek, T.G., Rollin, P.E., Nichol, S.T., 1995. Coexistence of several novel hantaviruses in rodents indigenous to North America. Virology 213, 122–130. https://doi.org/10.1006/viro.1995.1552.

Sandmann, S., Meisel, H., Ražanskienė, A., Wolbert, A., Pohl, B., Krüger, D.H., Sasnauskas, K., Ulrich, R., 2005. Detection of human hantavirus infections in Lithuania. Infection 33, 66–72. https://doi.org/10.1007/s15010-005-4058-8.

Saxenhofer, M., Weber de Melo, V., Ulrich, R.G., Heckel, G., 2017. Revised time scales of RNA virus evolution based on spatial information. Proc. R. Soc. B Biol. Sci. 284 https://doi.org/10.1098/rspb.2017.0857.

Saxenhofer, M., Schmidt, S., Ulrich, R.G., Heckel, G., 2019. Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus. PLoS Biol. 17, e3000142 https://doi.org/10.1371/journal.pbio.3000142.

Scharninghausen, J.J., Pfeffer, M., Meyer, H., Davis, D.S., Honeycutt, R.L., Faulde, M., 2002. Genetic evidence for Tula virus in *Microtus arvalis* and *Microtus agrestis* populations in Croatia. Vector Borne Zoonotic Dis. 2, 19–27. https://doi.org/10.1089/153036602760260742.

Schlegel, M., Kindler, E., Essbauer, S.S., Wolf, R., Thiel, J., Groschup, M.H., Heckel, G., Oehme, R.M., Ulrich, R.G., 2012a. *Tula* virus infections in the Eurasian water vole in

Central Europe. Vector Borne Zoonotic Dis. 12, 503–513. https://doi.org/10.1089/vbz.2011.0784.

Schlegel, M., Ali, H.S., Stieger, N., Groschup, M.H., Wolf, R., Ulrich, R.G., 2012b. Molecular identification of small mammal species using novel cytochrome B gene-derived degenerated primers. Biochem. Genet. 50, 440–447. https://doi.org/10.1007/s10528-011-9487-8.

Schlegel, M., Jacob, J., Krüger, D.H., Rang, A., Ulrich, R.G., 2014. Hantavirus emergence in rodents, insectivores and bats: what comes next? In: Johnson, N. (Ed.), The Role of Animals in Emerging Viral Diseases. Academic Press, Waltham, MA, pp. 235–292. https://doi.org/10.1016/C2011-0-09654-0.

Schmaljohn, C.S., 1990. Nucleotide sequence of the L genome segment of Hantaan virus. Nucleic Acids Res. 18, 6728. https://doi.org/10.1093/nar/18.22.6728.

Schmaljohn, C.S., Dalrymple, J.M., 1983. Analysis of Hantaan virus RNA: evidence for a new genus of Bunyaviridae. Virology 131, 482–491. https://doi.org/10.1016/0042-6822(83)90514-7.

Schmidt, S., Saxenhofer, M., Drewes, S., Schlegel, M., Wanka, K.M., Frank, R., Klimpel, S., von Blanckenhagen, F., Maaz, D., Herden, C., Freise, J., Wolf, R., Stubbe, M., Borkenhagen, P., Ansorge, H., Eccard, J.A., Lang, J., Jourdain, E., Jacob, J., Marianneau, P., Heckel, G., Ulrich, R.G., 2016. High genetic structuring of Tula hantavirus. Arch. Virol. 161, 1135–1149. https://doi.org/10.1007/s00705-016-2762-6.

Schmidt-Chanasit, J., Essbauer, S., Petraityte, R., Yoshimatsu, K., Tackmann, K., Conraths, F.J., Sasnauskas, K., Arikawa, J., Thomas, A., Pfeffer, M., Scharninghausen, J.J., Splettstoesser, W., Wenk, M., Heckel, G., Ulrich, R.G., 2010. Extensive host sharing of central European Tula virus. J. Virol. 84, 459–474. https://doi.org/10.1128/jvi.01226-09.

Sironen, T., Plyusnin, A., 2011. Genetics and evolution of hantaviruses. In: Plyusnin, A. E., Elliott, R.M. (Eds.), Bunyaviridae: Molecular and Cellular Biology. Caister Academic Press, Norfolk, UK, pp. 61–94.

Song, J.W., Gligic, A., Yanagihara, R., 2002. Identification of Tula hantavirus in *Pitymys subterraneus* captured in the Cacak region of Serbia-Yugoslavia. Int. J. Infect. Dis. 6, 31–36. https://doi.org/10.1016/S1201-9712(02)90133-5.

Song, K.J., Baek, L.J., Moon, S., Ha, S.J., Kim, S.H., Park, K.S., Klein, T.A., Sames, W., Kim, H.C., Lee, J.S., Yanagihara, R., Song, J.W., 2007. Muju virus, a novel hantavirus

harboured by the arvicolid rodent *Myodes regulus* in Korea. J. Gen. Virol. 88, 3121–3129. https://doi.org/10.1099/vir.0.83139-0.

Song, W., Torrez-Martinez, N., Irwin, W., Harrison, F.J., Davis, R., Ascher, M., Jay, M., Hjelle, B., 1995. Isla vista virus: a genetically novel hantavirus of the California vole *Microtus californicus*. J. Gen. Virol. 76 (Pt 12), 3195–3199. https://doi.org/10.1099/0022-1317-76-12-3195.

Spiropoulou, C., 2011. Bunyaviridae: Molecular and Cellular Biology. Caister Academic Press, Norfolk, UK, pp. 41–60.

Straková, P., Jagdmann, S., Balčiauskas, L., Balčiauskienė, L., Drewes, S., Ulrich, R.G., 2017. Puumala virus in bank voles, Lithuania. Emerg. Infect. Dis. 23, 158–160. https://doi.org/10.3201/eid2301.161400.

Thomason, A.G., Begon, M., Bradley, J.E., Paterson, S., Jackson, J.A., 2017. Endemic hantavirus in field voles, Northern England. Emerg. Infect. Dis. 23, 1033–1035. https://doi.org/10.3201/eid2306.161607.

Turell, M.J., Korch, G.W., Rossi, C.A., Sesline, D., Enge, B.A., Dondero, D.V., Jay, M., Ludwig, G.V., Li, D., Schmaljohn, C.S., Jackson, R.J., Ascher, M.S., 1995. Short report: prevalence of hantavirus infection in rodents associated with two fatal human infections in California. Am. J. Trop. Med. Hyg. 52, 180–182. https://doi.org/10.4269/ajtmh.1995.52.180.

von Tast, J., 1982. *Microtus oeconomus* (Pallas, 1776) — Nordische Wühlmaus, Sumpfmaus. In: Niethammer, J., Krapp, F. (Eds.), Handbuch der Säugetiere Europas. Akademische Verlagsgesellschaft, Wiesbaden, Germany.

Weber de Melo, V., Sheikh Ali, H., Freise, J., Kuhnert, D., Essbauer, S., Mertens, M., Wanka, K.M., Drewes, S., Ulrich, R.G., Heckel, G., 2015. Spatiotemporal dynamics of Puumala hantavirus associated with its rodent host, *Myodes glareolus*. Evol. Appl. 8, 545–559. https://doi.org/10.1111/eva.12263.

Zou, Y., Wang, J.B., Gaowa, H.S., Yao, L.S., Hu, G.W., Li, M.H., Chen, H.X., Plyusnin, A., Shao, R., Zhang, Y.Z., 2008. Isolation and genetic characterization of hantaviruses carried by *Microtus* voles in China. J. Med. Virol. 80, 680–688. https://doi.org/10.1002/jmv.21119.

Žvirblienė, A., Samonskyte, L., Gedvilaitė, A., Voronkova, T., Ulrich, R., Sasnauskas, K., 2006. Generation of monoclonal antibodies of desired specificity using chimeric polyomavirus-derived virus-like particles. J. Immunol. Methods 311, 57–70. https://doi.org/10.1016/j.jim.2006.01.007.

Table S1 Primers used for RT-PCR screening and primer walking for Rusne virus genome sequence determination and generation of the entire N protein sequence for expression.

Name	Sequence 5′→3′	Genome segment	Reference
NCRS	TAGTAGTAGACTCCTTGAA	S	Ali et al., 2015
RuS1REV	ATTCCTAACCTGTATTTGTG	S	This study
Pu342F	TATGGTAATGTCCTTGATGT	S	Essbauer et al., 2006
Pu1102R	GCCATDATDGTRTTYCTCAT	S	Essbauer et al., 2006
RuS3FW	TAGATAAAAACCATATTCCTGAT	S	This study
RuS3REV	ATTCCTATTACATGACCTAAC	S	This study
RuS4FW	TATCACTTAATATAGCCATTAG	S	This study
RuS4FW_b	TATATATGTTATTCAGGTCATC	S	This study
RuSLIGFW	GCAGCTTTCCCAGCAATATGT	S	This study
RuSLIGREV	GCCAGTTTATCCTCCAGTGCT	S	This study
RuM1FW	TAGTAGTAGACTCCGCA	M	Ali et al., 2015; modified
RuM1REV	TATCCATATCATGATCTTCC	M	This study
RuM2FW	GACTATAGATCTTCAGAGATTTT	M	This study
RuM2REV	AGTAACTTGAACTTTGAATCT	M	This study
RuM3FW	AGAAAGTTATCCTTACAAAG	M	This study
RuM3REV	GTATTTTCTCTTGTTCATTGG	M	This study
RuM4FW	AAAAGTGACTTAGAGCTAGA	M	This study
RuM4REV	AGTATACGATCCTACCATCC	M	This study
RuM5FW	ATGGATAGTTGGAATTTTAAG	M	This study
Han_Uni_M1	TAGTAGTAKRCWCCGCA	M	Ali et al., 2015
Han LF1	ATGTAYGTBAGTGCWGATGC	L	Klempa et al., 2006
Han LR1	AACCADTCWGTYCCRTCATC	L	Klempa et al., 2006
RuL1FW	TAGTAGTAGACTCCGAGAGAGAGAA	L	This study
RuL1REV	TCTCTCACTCCTCTAGCCAC	L	This study
RuL2FW	TCCCAGGAGAGACATCCGC	L	This study
RuL2REV	GCAGTGGTGTCTCTGGGTAAT	L	This study
RuL3FW	CATACCTGATTATCGCCCACAAGT	L	This study
RuL3REV	CAGCAACTTCTAATGATTTRGAKGG	L	This study
RuL4FW	AAAACTGCTGCATGGCATCTC	L	This study
RuL4REV	CTGATGCCCATCTCAATGCC	L	This study
RuL5FW	TGTCAGGATCATTGCAGGAAGA	L	This study
RuL5REV	GCAGCACCGAACAATGAAGA	L	This study
RuL6FW	GTATGGACCAATTTATTCCCTGAG	L	This study
RuL6REV	CACCCCCTAAAGGGATTGGG	L	This study
RuL7FW	GCTGTGCTGTCTCAATACCCT	L	This study
RuL7REV	GGATGTTGCTCAGTCCAAGC	L	This study
RuL8FW	TGGCAACAGCAGGTATTGGT	L	This study
RuL8REV	TGTCCTGGCAATCTTCGGTC	L	This study

Table S1 (continued)

Name	Sequence 5′→3′	Segment	Reference
RuL9FW	CCGCCATGACAATGCAATCA	L	This study
RuL9REV	TGATCGTTAGCCTTGCACCA	L	This study
RuL10FW	TGGCAACAGCAGGTATTGGT	L	This study
RuL10REV	ACGCAGACCTGTCCGTAGA	L	This study
RuL11FW	GGGGGTACTACCAGTTGATCC	L	This study
RuL11REV	CTTTCTGCTGGGCCTCTTGAT	L	This study
RuL12FW	TTGGCATTGACTGTGCGAGA	L	This study
RuL12REV	CCTCAGACCATTGTGTACTAGA	L	This study
RuL13FW	TTACCTGCTGCAATCCCTCC	L	This study
RuL13REV	CCCTGTTATCTTGTAATTATCAGG TG	L	This study
RuLLIGFW	CCAGCAGAAAGTTATGGAGGAAA AAA	L	This study
RuLLIGREV	CCCTGTTATCTTGTAATTATCAGG TG	L	This study
RuNRES_FOR	AAATCTAGAAGCAACCTCAAAGA AATACAAGAG	S	This study
RuNRES_REV	TTTTCTAGATTATATCTTAAGTGG TTCCTGGTT	S	This study

FW, forward primer; REV, reverse primer; LIGFW and LIGREV, RNA ligation primer forward and reverse, RuNRES_FOR and RuNRES_REV, primers for amplification of nucleocapsid protein coding sequence with XbaI restriction sites (underlined) used for insertion into yeast expression plasmid

Table S2 RT-PCR and ELISA results for root voles (*Microtus oeconomus*) that were positive in at least one assay.

ID	Trapping site	Date of capture	Sex	Age	Mass (g)	RT-PCR	IgG ELISA	Accession numbers
LT15/299	Rusnė	23.-25.09.2015	male	adult	46.5	pos	neg	MT441731 (S) MT441736 (M) MT441739 (L)
LT15/301	Rusnė	23.-25.09.2015	female	adult	42.5	pos	pos	MT441733 (S) MT441737 (M) MT441740 (L)
LT15/306	Rusnė	23.-25.09.2015	male	adult	53	pos	pos	(S)**
LT15/341	Rusnė	23.-25.09.2015	male	juvenile	20	pos	neg	(S), (M), and (L)***
LT15/351	Rusnė	23.-25.09.2015	female	subadult	18.5	pos	Pos	MT441732 (S) MT441738 (M) MT441741 (L)***
LT15/399	Rusnė	23.-25.09.2015	male	adult	44	pos	Pos	MT441734 (S)
LT16/020-Ru	Rusnė	11.-13.11.2016	male	adult	48.5	pos	Neg	(S)**
LT17/R6*	Rusnė	16.-18.10.2017	male	adult	35	pos	Neg	MT441735* (S)

pos, positive; neg, negative; S, S segment; M, M segment; L, L segment

*Sample was used to generate the recombinant Rusne virus nucleocapsid protein antigen.

**Sequence identical to sequence of strain LT15/399.

***Sequences of strain LT15/341 were complete for S and M CDS and incomplete for L CDS; these sequences are identical to those of strain LT15/351.

70

Table S3 Accession numbers for hantavirus sequences used in Fig. 3 and Fig. S2.

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Altai hantavirus				KM361055-KM361057	KM361055-KM361057
Andes orthohantavirus	AF004660, AF325966, AF482713-AF482717, AY228237, DQ345763, EF571895, KY659432, NC_003466, KP202360	AF004660, AF325966, AF482713-AF482717, AY228237, DQ345763, EF571895, KY659432, NC_003466, KP202360	AF028022, AF028024, AF324901, AF291703, AY228238, KY604962	AF291704, JX443698	AF291704, JX443698
Asama orthohantavirus	EU929070, EU929071	EU929070, EU929071	EU929073, EU929075		
Asikkala orthohantavirus	KC880341-KC880343	KC880341-KC880343	KC880344, KC880345		
Bayou orthohantavirus	NC_038298	NC_038298	GQ244521, L36930	FJ858378, GQ244526	FJ858378, GQ244526
Black Creek Canal orthohantavirus	L39949	L39949	L39950	GU997097	GU997097
Bowe orthohantavirus	NC_034405	NC_034405	NC_034406	KC631784	KC631784
Bruges orthohantavirus	MF683844	MF683844	MF683845	KX551962	KX551962
Camp Ripley hantavirus				KF958465	KF958465
Cano Delgadito orthohantavirus	NC_034528	NC_034528	NC_034525	GQ200821	GQ200821
Cao Bang orthohantavirus	JX465405, JX465406, JX465409, JX465410, KJ162406, NC_034484	JX465405, JX465406, JX465409, JX465410, KJ162406, NC_034484	KJ162397, NC_034474	EF543525, KF705679	EF543525, KF705679
Catacamas hantavirus				JX443698	JX443698
Choclo orthohantavirus	DQ285046, KM597161, KT983771	DQ285046, KM597161, KT983771	DQ285047, KT983772	EF397003, KT983773	EF397003, KT983773

Table S3 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Dobrava-Belgrade orthohantavirus	AJ009773, AJ009775, AJ269549, AJ269550, AJ131672, AJ131673, AJ410619, AY168576, AY961618, AY533120, EU188449-EU188452, GQ205401-GQ205402, GQ205404-GQ205407, GU904027-GU904028, JF920150, JQ026204, KT885043, NC_005233	AJ009773, AJ009775, AJ269549, AJ269550, AJ131672, AJ131673, AJ410619, AY168576, AY961618, AY533120, EU188449-EU188452, GQ205401-GQ205402, GQ205404-GQ205407, GU904027-GU904028, JF920150, JQ026204, KT885043, NC_005233	AJ009774, AY168577, AY168578, AY961616, GQ205409, GQ205411, GU904033, GU904034, JQ026205, KT885042, L33685, Q205409, Y168577	AJ410617, JF920148, JQ026206	AJ410617, JF920148, JQ026206
El Moro Canyon orthohantavirus	NC_034396	NC_034396			
Fugong orthohantavirus	KT899701	KT899701	NC_034466	KT899703	KT899703
Fusong orthohantavirus	EU072480, EU072481, FJ170792, FJ170794, FJ170796, FJ170797	EU072480-EU072481, FJ170792, FJ170794, FJ170796, FJ170797	EU072488, KJ857333	KJ857316	KJ857316
Hantaan orthohantavirus	AB620028, AB620031, AF427318, AF427319, AF427322, AF427323, KJ857347, KJ857348	AB620028, AB620031, AF427318, AF427319, AF427322, AF427323, KJ857347, KJ857348	AB027115, EU363815, FJ753399, JQ665881, JQ665882, JQ912804, KP970560, KU207203, KU207204, L08753, Y00386	AB620030, AF189155, AF285266, AF288292, AF288297, AF293665, AY675354, D25528, D25531, DQ056292, KC136242, KJ857317, X55901	AB620030, AF189155, AF285266, AF288292, AF288297, AF293665, AY675354, D25528, D25531, DQ056292, KC136242, KJ857317, X55901
Imjin thottimvirus	EF641804, EF641805, KJ420559	EF641804, EF641805, KJ420559	EF641797-EF641799, KX779127, NC_034557		
Isla Vista hantavirus	U19302, U31534, U31535	U19302, U31534, U31535			
Jeju orthohantavirus	HQ663933, HQ834695	HQ663933, HQ834695	NC_034404	HQ663935, HQ834697	HQ663935, HQ834697
Kenkeme orthohantavirus	GQ306148, MG279218, NC_034559	GQ306148, MG279218, NC_034559	NC_034565	KJ857320	KJ857320

Table S3 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Khabarovsk orthohantavirus	AJ011646, KJ857343-KJ857346, NC_034527	AJ011646, KJ857343-KJ857346, NC_034527	AJ011647, EU072489, KJ857329, KJ857330, KJ857332, KJ857339, KJ857340, NC_034518	KJ857311-KJ857314, KJ857322	KJ857311-KJ857314, KJ857322
Kielder hantavirus				KY751731-KY751732	
Kilimanjaro hantavirus				JX193700	JX193700
Laguna Negra orthohantavirus	AF005727, KP202359	AF005727, KP202359	NC_038506	FJ809772, JQ6111713, JX443696, JX443697	FJ809772, JQ6111713, JX443696, JX443697
Laibin mobatvirus	KM102247, KY662264	KM102247, KY662264	KM102248, KY662265		
Longquan loanvirus	JX465413	JX465413	JX465397, JX465399-JX465401		
Luxi orthohantavirus				HQ404253, KT899703	HQ404253
Maporal orthohantavirus				EU788002	EU788002
Montano orthohantavirus	NC_034396	NC_034396	NC_034397		
Nova mobatvirus	KT004445, KX512328, KX512330, KX512345	KT004445, KX512328, KX512330, KX512345	KT004446, KX512426-KX512430, KY780087, NC_034470	AB620102, AB620105, AB620108	AB620102, AB620105, AB620108
Necocli orthohantavirus	KF481954	KF481954	KF494345		
Oxbow orthohantavirus	FJ539166	FJ539166	FJ539167		
Prospect Hill orthohantavirus	NC_038938, M34011, U19303, U47136	NC_038938, M34011, U19303, U47136	X55129	EF646763	EF646763

Table S3 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Puumala orthohantavirus	AB010730, AB297665, AB433843, AB433845, AB675463, AB675474, AB675478, AB677476- AB677477, AF367064- AF367066, AF367068, AF367069-AF367071, AF442613, AJ223368- AJ223369, AJ223371, AJ223374 - AJ223377, AJ223380, AY526219, DQ138140, DQ138142, EF211819- EF211820, EF442087, GQ339473, GQ339477- GQ339481, GQ339483-GQ339484, GQ339487, JN657228, JX028273, KT885052, KX815394, M32750, U14137, Z21497, Z30706-Z30708, Z84204	AB010730, AB297665, AB433843, AB433845, AB675463, AB675474, AB677478, AB677476- AB677477, AF367064- AF367066, AF367068, AF367069-AF367071, AF442613, AJ223368- AJ223369, AJ223371, AJ223374, AJ223377, AJ223380, AY526219, DQ138140, DQ138142, EF211819-EF211820, EF442087, GQ339473, GQ339477-GQ339481, GQ339483-GQ339484, GQ339487, JN657228, JX028273, KT885052, KX815394,M32750, U14137, Z21497, Z30706-Z30708, Z84204	AB297666, AB433850, AB433852, AF442615, AF442616, AB676848, AY526218, EF422372, HE801634, JN831944, JQ319172, JQ319174, JQ319175, JX046486, L08755, M29979, NC_005223, Z84205, Z49214	AB297667, AB574183, AB574184, AB712372, AY526217, EF405801, HE801635, JN831946, JN831949, JN831952, JX028271, JX046482, JX046485, KJ994778, KT885050, M63194	AB297667, AB574183, AB574184, AB712372, AY526217, EF405801, HE801635, JN831946, JN831949, JN831952, JX028271, JX046482, JX046485, KJ994778, KT885050, M63194
Quezon mobatvirus	KU950713	KU950713	NC_034393		
Rockport orthohantavirus	HM015218, HM015223- HM015224	HM015218, HM015223- HM015224	HM015219	HM015220-HM015222	HM015220-HM015222
Sangassou orthohantavirus	JQ082303, NC_034526	JQ082303, NC_034526	NC_034516		
Seewis orthohantavirus	EF636024, KF974360, KM201411	EF636024, KF974360, KM201411	KF974359		

Table S3 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Seoul orthohantavirus	AF288653, AF288655, AF329388, AF329389, EF192308, FJ803205-FJ803208, FJ803210, FJ803211, FJ803213-FJ803216, GQ274945, GQ279392, GU361893, GU592938, GU592941-GU592946-GU592944, GU592946-GU592949, GU592951, GU592953, GU361893, JQ665912, JQ898106, JQ912773, JQ912775-JQ912777, JQ912779, KF387725, KM948598, KP859511, MF149948	AF288653, AF288655, AF329388, AF329389, EF192308, FJ803205-FJ803208, FJ803210, FJ803211, FJ803213-FJ803216, GQ274945, GQ279392, GU361893, GU592938, GU592941-GU592946-GU592944, GU592946-GU592949, GU592951, GU592953, GU361893, JQ665912, JQ898106, JQ912773, JQ912775-JQ912777, JQ912779, KF387725, KM948598, KP859511, MF149948	AF145977, D17592, GU592924-GU592926, GU592929, GQ274943, JQ665888, JX879768, MF149945, N377554, X53861	AF285266, AF288297	AF285266, AF288297
Sin Nombre orthohantavirus	JQ690277, JQ690278, JQ690282, KT885046, L37904, NC_005216	JQ690277, JQ690278, JQ690282, KT885046, L37904, NC_005216	AF030551, AF030552, JQ690279, JQ690280, JQ690283, JQ690284, KT885045, L37903, NC_005215	AF425256, KT885044, L37901, MG717393, MH539865	AF425256, KT885044, L37901, MG717393, MH539865
Tatenale hantavirus	JX316009, MK883756, MK883757, MN267822	MN267822, MK883756, MK883757,	MK883759	JX316008, MK883760, MK883761, MN267824	MK883760MK883761
Thailand orthohantavirus	AB186420, AM397664, AM998808, GQ274941, KC490915, KC490916, KC490918, M998808	AB186420, AM397664, AM998808, GQ274941, KC490915, KC490916, KC490918, M998808	L08756, NC_034563	KC490922, KU587796	KC490922, KU587796
Tigray orthohantavirus				KU934008	KU934008
Thottapalayam thottimvirus	JF784172, KJ420560	JF784172, KJ420560	DQ825771, EU001329 JF784178-JF784180, KJ420542-KJ420545		
Traemmersee hantavirus	MK542662	MK542662	MK542663	MK542664	MK542664

Table S3 (continued)

Hantavirus	S partial	S CDS	M CDS	L partial	L CDS
Tula orthohantavirus	AF017659, AF063892, AF164093, AF164094, AF164097, AF289819-AF289821, AF442621, AJ223600, AJ223601, AM945877, EU439949-EU439951, KP013568, KP013572, KT946591, KU139529-KU139531, KU139533, KU139535, KU139537-KU139539, KU139549, KU139553, KU139555-KU139557, KU139558, KU139563, KU139565-KU139566, KU139569-KU139570, KU139577-KU139579, KU139595-KU139597, KU139600, KU139605, MK386130-MK386141 MK535072, MK535084, MK535086, NC_005227, Y13979, Y13980, Z30941-Z30945, Z48741, Z48574, Z69991	AF017659, AF063892, AF164093, AF164094, AF164097, AF289819-AF289821, AF442621, AJ223600, AJ223601, AM945877, EU439949-EU439951, KP013568, KP013572, KT946591, KU139529-KU139531, KU139533, KU139535, KU139537-KU139539, KU139549, KU139553, KU139555-KU139557, KU139558, KU139563, KU139565-KU139566, KU139569-KU139570, KU139577-KU139579, KU139595-KU139597, KU139600, KU139605, MK386130-MK386141 MK535072, NC_005227, Y13979, Y13980, Z30941-Z30945, Z48741, Z48574, Z69991	NC_005228	AJ005637, MK386130-MK386140, MK535003-MK535005, MK535007-MK535011, MK535013-MK535018, MK535020-MK535024, MK535027-MK535029, MK535030, MK535032-MK535037	AJ005637
Uluguru hantavirus				JX193697	JX193697
Xinyi hantavirus				KF705679	KF705679
Xuan Son hantavirus			KY662268, KY662271	KY662269, KY662272, KY662275	KY662269, KY662272, KY662275
Yakeshi orthohantavirus	NC_038704	NC_038704	JX465403		

Table S4 Pairwise comparison of partial nucleotide sequences of the S and L segment, and amino acid sequences of the nucleocapsid (N) protein and RNA-dependent RNA polymerase (RdRP) of Rusne virus strain LT15/301, other Rusne virus strains and reference sequences. Pairwise comparison of partial nucleotide (nt) and amino acid (aa) sequences of the S segment (nt 622-1015, numbering according Khabarovsk orthohantavirus, GenBank accession number NC_034527) and the N protein (aa residues 194-324) of the novel root vole-associated Rusne virus strain LT15/301 to other Rusne virus strains and the most related reference sequences identified by Basic Local Alignment Search Tool (BLAST) and of partial nt and aa sequences of the L segment (nt 2962-3324, according to Khabarovsk orthohantavirus, GenBank accession number NC_034519) and the RdRP (aa residues 976-1096). All sequences are shown that have at least a nt sequence identity of 0.75 in either partial S or partial L segments.

	S segment / N protein		L segment / RdRP	
Reference sequence	nt	aa	nt	aa
Rusne virus LT15/299 MT441731, MT441739	1	1	1	1
Rusne virus LT15/351 MT441732, MT441741	1	1	0.996	1
Rusne virus LT15/399 MT441734	0.997	1	n.a.	n.a.
Rusne virus LT17/R6 MT441735	0.987	1	n.a.	n.a.
Traemmersee virus K542662, MK542664	0.809	0.977	0.837	0.963
Tatenale virus 2016 MN267822, MN267824	0.796	0.954	0.831	0.99
Tatenale virus JX316009; JX316008	0.798	0.954	0.834	0.981
Tatenale virus strain Norton Juxta MK883757, MK883761	0.801	0.954	0.822	0.981
Tatenale virus strain Upton Heath MK883756, MK883760	0.796	0.961	0.825	0.99
Kielder virus KY751731	n.a.	n.a.	0.819	0.99
Kielder virus KY751732	n.a.	n.a.	0.816	0.981
Khabrovsk strain Topografov virus AJ011646	0.773	0.854	n.a.	n.a.
Khabarovsk virus NC_034527, KJ857311	0.743	0.847	0.804	0.918

Table S4 (continued)

	S segment / N protein		L segment / RdRP	
Reference sequence	nt	aa	nt	aa
Fusong strain Yuanjiang virus FJ170792, KJ857316	0.773	0.824	0.759	0.882
Fusong strain Shenyang virus FJ170796	0.745	0.801	n.a.	n.a.
Fusong virus EU072480	0.748	0.809	n.a.	n.a.
Puumala virus strain Muju JX028273, JX046485	0.763	0.778	0.768	0.882
Puumala virus strain Hokkaido AB675474, AB712372	0.732	0.786	0.771	0.864
Puumala virus strain Ussuri Khekhtsir AB677476	0.745	0.778	n.a.	n.a.
Puumala like Fusong virus EF211820	0.727	0.77	n.a.	n.a.
Fugong virus KT899701, KT899703	0.558	0.573	0.762	0.837
Catacamas virus FJ858378	n.a.	n.a.	0.75	0.855
Prospect Hill virus M34011, EF646763	0.674	0.709	0.762	0.891
Puumala virus AF367066, EF405801	0.74	0.763	0.78	0.864
Tula virus NC_005227, AJ005637	0.656	0.648	0.744	0.882

n.a., sequence not available

Table S5 Pairwise comparison of complete nucleotide (nt) sequences of the S, M and L segment and amino acid (aa) sequences of the nucleocapsid (N) protein, glycoprotein precursor (GPC) and RNA-dependent RNA polymerase (RdRP) of Rusne virus strain LT15/301, other Rusne virus strains and reference sequences, as well as pairwise evolutionary distances (PED). Reference sequences were selected that reached a nucleotide sequence identity of at least 0.75 in one of the three segments.

Reference sequence	S / N protein		M / GPC		L / RdRP		PED
	nt	aa	nt	aa	nt	aa	
Rusne virus LT15/299 MT441731, MT441736, MT441739	1	1	0.998	0.996	0.998	0.999	n.d.
Rusne virus LT15/351 MT441732, MT441738, MT441741	1	1	0.996	0.999	0.995	0.998	n.d.
Rusne virus LT15/399 MT441734	0.997	1	n.a.	n.a.	n.a.	n.a.	n.d.
Rusne virus LT17/R6 MT441735	0.990	1	n.a.	n.a.	n.a.	n.a.	n.d.
Traemmersee virus MK542662, MK542663, MK542664	0.841	0.976	0.809	0.939	0.815	0.953	0.05204
Tatenale virus strain Norton Juxta MK883757, MK883759, MK883761	0.847	0.972	0.802	0.939	0.806	0.946	0.05261
Tatenale virus strain Upton Heath MK883756, MK883758, MK883760	0.844	0.974	0.811*	0.937*	0.809	0.948	n.d.

Table S5 (continued)

Reference sequence	S / N protein		M / GPC		L / RdRP		PED
	nt	aa	nt	aa	nt	aa	
Tatenale virus 2016 MN267822, MN267823, MN267824	0.837	0.972	0.796*	0.932*	0.802*	0.938*	n.d.
Fugong virus KT899701, NC_034466	0.704	0.747	0.753	0.713	n.a.	n.a.	0.33366
Fusong virus EU072480, EU072488	0.773	0.879	0.699	0.847	n.a.	n.a.	0.15519
Khabarovsk virus strain Topografov AJ011646, AJ011647	0.788	0.886	0.744	0.842	n.a.	n.a.	0.15551
Fusong strain Yuanjiang virus FJ170792, KJ857333, KJ857316	0.784	0.882	0.76	0.856	0.76	0.879	0.14696
Khabarovsk virus NC_034527, NC_034518, KJ857315	0.776	0.882	0.758	0.858	0.759	0.88	0.14306
Puumala virus AJ223376, NC_005223, HE801635	0.778	0.856	0.742	0.819	0.757	0.86	0.18414
Tula virus NC_005227, NC_005228, AJ005637	0.749	0.796	0.736	0.818	0.739	0.846	0.20751

n.a., sequence not available

n.d., not determined

* only partial, but almost complete CDS available, for M and L sequence information on the 5′ end of the CDS is missing including the start codon

Table S6

Genome organization and coding capacity of Rusne virus strain LT15/301 and other Arvicolinae-associated orthohantaviruses.

Orthohantavirus	Genome (length in nt) 5'-NCR, CDS, 3'-NCR			Protein (size in aa)			
	S	M	L	N	NSs	GPC	RdRP
Rusne virus LT15/301 MT441733, MT441737, MT441740	42, 1302, 715	28*, 3447, 214*	24*, 6465, 31*	433	94	1148	2154
Traemmersee virus MK542662, MK542663, MK542664	n.a., 1302, n.a.	n.a., 3447, n.a.	n.a, 6465, n.a.	433	90	1148	2154
Tatenale virus 2016 MN267822, MN267823, MN267824	42, 1302, 650	n.a., 3408*, 92	n.a, 6421*, 25.	433	90	1135*	2139*
Tatenale virus strain Norton Juxta MK883757, MK883759, MK883761	n.a., 1302, n.a.	n.a., 3447, n.a.	n.a, 6465, n.a	433	90	1148	2154
Tatenale virus strain Upton Heath MK883756, MK883757, MK883760	n.a., 1302, n.a.	n.a., 3355*, n.a.	n.a, 6465, n.a.	433	90	1118*	2154
Fugong virus KT899701, NC_034466	42, 1308, 463	58, 3447, 152	n.a., n.a., n.a.	435	69	1139	n.a.

Table S6 (continued)

Orthohantavirus	Genome (length in nt) 5'-NCR, CDS, 3'-NCR			Protein (size in aa)			
	S	M	L	N	NSs	GPC	RdRP
Fusong virus EU072480, EU072488	42, 1293, 499	n.a., 3458, n.a.	n.a, n.a, n.a.	433	77	1145	n.a.
Khabarovsk virus strain Topografov AJ011646, AJ011647	42, 1302, 607	49, 3435, 222	36, 6465, 78	433	95	1144,	2154
Fusong virus strain Yuanjiang FJ170792, KJ857333, KJ857316	42, 1302, n.a.	n.a., 3438, 197	36, 6471, n.a.	433	95	1145	2156
Khabarovsk virus NC_034527, NC_034518, KJ857315	42, 1302, 501	49, 3435, 222	36, 6465, 78	433	95	1144	2154
Puumala virus AJ223376, NC_005223, HE801635	42, 1302, 527	40, 3447, 195	36, 6471, 43	433	90	1148	2156
Tula virus NC_005227, NC_005228, AJ005637	42, 1293, 499	55, 3426, 213	36, 6462, 43	430	90	1141	2153

n.a. not available; CDS, coding sequence; NCR, non-coding region
* partial sequence

References

Ali, H.S., Drewes, S., Weber de Melo, V., Schlegel, M., Freise, J., Groschup, M.H., Heckel, G., Ulrich, R.G., 2015. Complete genome of a Puumala virus strain from Central Europe. Virus Genes 50, 292-298. https://doi.org/10.1007/s11262-014-1157-6.

Essbauer, S., Schmidt, J., Conraths, F.J., Friedrich, R., Koch, J., Hautmann, W., Pfeffer, M., Wölfel, R., Finke, J., Dobler, G., Ulrich, R., 2006. A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. Epidemiol. Infect. 134, 1333-1344. https://doi.org/10.1017/s0950268806006170.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Denys, C., Koivogui, L., ter Meulen, J., Krüger, D.H., 2006. Hantavirus in African wood mouse, Guinea. Emerg. Infect. Dis. 12, 838-840. https://doi.org/10.3201/eid1205.051487.

Fig. S1

Fig. S2

3.3   Paper III

Jeske K, Emirhar D, Garcia JT, González-Barrio D, Olea PP, Ruiz Fons F, Schulz J, Mayer-Scholl A, Heckel G, Ulrich RG. Multiple *Leptospira* spp. detection, but absence of Tula orthohantavirus in *Microtus* voles, Northwestern Spain. Journal of Wildlife Diseases (submitted)

FREQUENT *LEPTOSPIRA* SPP. DETECTION BUT ABSENCE OF TULA ORTHOHANTAVIRUS IN *MICROTUS* SPP. VOLES, NORTHWESTERN SPAIN

Kathrin Jeske,[1,2] **Duygu Emirhar,**[3] **Jesús T. García,**[4] **David González-Barrio,**[5] **Pedro P. Olea,**[6,7] **Francisco Ruiz Fons,**[4] **Jana Schulz,**[1,8] **Anne Mayer-Scholl,**[3] **Gerald Heckel,**[9] **and Rainer G. Ulrich**[1,10]

[1] Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald–Insel Riems, Germany

[2] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany

[3] Department for Biological Safety, Federal Institute for Risk Assessment, Diedersdorfer Weg 1, 12277 Berlin, Germany

[4] Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo 12, 13071 Ciudad Real, Spain

[5] SALUVET, University Complutense (UCM), Av. Puerta de Hierro, 28049 Madrid, Spain

[6] Departamento de Ecología, Universidad Autónoma de Madrid (UAM), C/Darwin 2, 28049 Madrid, Spain

[7] Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM), Universidad Autónoma de Madrid, C/Darwin 2, Madrid, Spain

[8] Institute of Epidemiology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald–Insel Riems, Germany

[9] Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

[10] Corresponding author (email: RainerGuenter.Ulrich@fli.de)

ABSTRACT: The common vole (*Microtus arvalis*) is a major agricultural pest in Europe and is a reservoir for several zoonotic agents, such as *Leptospira* spp. and Tula orthohantavirus (TULV). However, little is known about the occurrence of those pathogens in voles from Spain, where the species has largely expanded its distribution range in the past decades, causing agricultural pests and zoonotic diseases. For a molecular survey, 580 common voles and six Lusitanian pine voles (*Microtus lusitanicus*) were collected in 26 localities from four provinces of northwestern Spain. We assessed the presence of *Leptospira* spp. DNA in kidney tissue by PCR targeting the *lipL32* gene, detecting a prevalence of 7.9% (95% confidence interval, 5.9–10.4) for common voles and of 33.3% (95% confidence interval, 4.3–77.7) for Lusitanian pine voles. We identified *Leptospira kirschneri* in 24 animals and *Leptospira borgpetersenii* in two animals, using *secY* gene–specific PCR. We analyzed environmental and demographic factors (such as age class, weight, and sex) and population dynamics data for their potential effect on the *Leptospira* spp. prevalence in those voles. The *Leptospira* spp. DNA detection rate in common voles increased significantly with maximum air temperature, vole weight, and amount of accumulated rainfall during the 90 d before capture and within the peak phase of the population cycle. We assessed the presence of TULV in lung tissue of 389 voles by reverse-transcription PCR, with no positive results. The absence of TULV might be explained by the evolutionary isolation of the common vole in Spain. The detection of two *Leptospira* genomospecies underlines the necessity for further typing efforts to understand the epidemiology of leptospiral infection in the common vole and the potential risk for human health in Spain.

Key words: age, common vole, hantavirus, *Leptospira* spp., Spain, weight, zoonoses.

INTRODUCTION

The common vole (*Microtus arvalis*) is broadly distributed in major parts of Europe and features massive population explosions in certain regions of central and western Europe (Jacob and Tkadlec 2010). These population booms can be associated with human leptospirosis and tularemia disease clusters (Desai et al. 2009; Luque-Larena et al. 2015). Leptospirosis, considered a (re)-emerging zoonotic disease in humans, is caused by pathogenic species of gram-negative bacteria of the genus *Leptospira*, such as *Leptospira kirschneri*, *Leptospira borgpetersenii*, and *Leptospira interrogans*. The main means of spread within one host species, be it rodent or livestock, is by direct transmission within their nests or territories (Faine et al. 1999). Zoonotic infection with these pathogenic *Leptospira* spp. results from direct or indirect exposure to carrier animals that shed the bacteria in their urine. The spectrum of

1

human disease is variable and can range from subclinical infections to fatal multiorgan dysfunction (Karpagam and Ganesh 2020). Rodents have recently been linked, directly or through epidemiological studies, to leptospirosis outbreaks in livestock (Fávero et al. 2017; Marquez et al. 2019). The transmission depends on several factors, including climatic conditions, agricultural and livestock system factors, and the natural range of movement of the animals (Mwachui et al. 2015).

Additionally, the common vole is the main reservoir of Tula orthohantavirus (TULV), which is broadly distributed in various parts of Europe, with low to moderate prevalence (Schmidt al. 2016; Maas et al. 2017). Only a few cases of human disease have been reported so far, with high fever, diffuse pain, headache (Reynes et al. 2015), renal syndrome, and pneumonia (Klempa et al. 2003), as well as dyspnea in an immunocompromised patient (Zelená et al. 2013). Hantaviruses, including TULV, are enveloped, with a segmented, negative-stranded RNA genome (Elliott 1990, Kukkonen et al. 1998). The small (S) segment and the large (L) segment encode the nucleocapsid protein and the RNA-dependent RNA polymerase, respectively, and are frequently used for molecular diagnostics and phylogenetic investigations (Sibold et al. 1999; Nikolic et al. 2014). Phylogenetic analysis of TULV sequences indicated a strong genetic structuring that is partially explained by their association to different evolutionary lineages of the common vole (Saxenhofer et al. 2017, 2019; Hiltbrunner and Heckel 2020).

There is no previous information about the infection with *Leptospira* and TULV in voles from Spain. However, pathogenic leptospires have been detected in other rodents in Spain (Arent et al. 2017; Millan et al. 2018), and hantavirus-reactive antibodies have been detected in humans and red foxes (*Vulpes vulpes*; Sanfeliu et al. 2011; Lledó et al. 2020). Therefore, our molecular survey aimed to evaluate the presence of *Leptospira* spp. and hantaviruses in voles from northwestern Spain, where the common vole has enlarged its distribution range in the past decades

(García et al 2020), and to identify potential factors related to the presence of these pathogens.

MATERIALS AND METHODS

Between 2011 and 2014, voles were trapped at 26 sampling sites in four provinces of northwestern Spain within the western part of the Duero River basin (Fig. 1 and Supplementary Material Table S1). For each animal, trapping data and site, including phase of the population cycle and distance to the nearest water point (in meters), species, weight, age class (determined by the classification scheme of Morris [1972]), sex, and various biometric measurements were recorded; further details of trapping, phases of the population cycle, necropsy and sample collection, and storage and shipment are provided in the Supplementary Material). All handling procedures were approved by the UCLM Ethics Committee (reference no. CEEA: PR20170201) and are in accordance with the Spanish and European policy for animal protection and experimentation.

For detection of pathogenic *Leptospira* spp., DNA was extracted from kidney samples using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Samples of DNA were initially analyzed by conventional *lipL32* PCR (Mayer-Scholl et al. 2011). All PCR-positive samples were then subjected to *secY* sequence typing (Victoria et al. 2008) followed by multilocus sequence typing (MLST) of selected *secY* PCR-positive samples (Boonsilp et al. 2013). We used a mitochondrial cytochrome *b* gene-specific PCR (Schlegel et al. 2012a) to identify or confirm vole species and as a control for the quality of the nucleic acid preparation.

For hantavirus detection, RNA was extracted from lung samples with QIAzol reagent (Schmidt et al. 2016) and validated by β-actin reverse transcription (RT)-PCR (Wakeley et al. 2005). The RNA samples were screened by conventional S segment–specific RT-PCR (Schmidt et al. 2016) and SYBR green–based L segment RT real-time PCR (RT-rtPCR; Schlegel et al. 2012b) using the QuantiTect SYBR Green RT-PCR Kit (QIAGEN).

We used univariate and multivariable generalized linear mixed-effects models (GLMMs) to investigate the influence of several factors, including climatic, common vole individual factors, and population cycle-phase information (see Supplementary Material Table S2) on individual test status for *lipL32* PCR-positive or -negative common voles (binomial response variable).
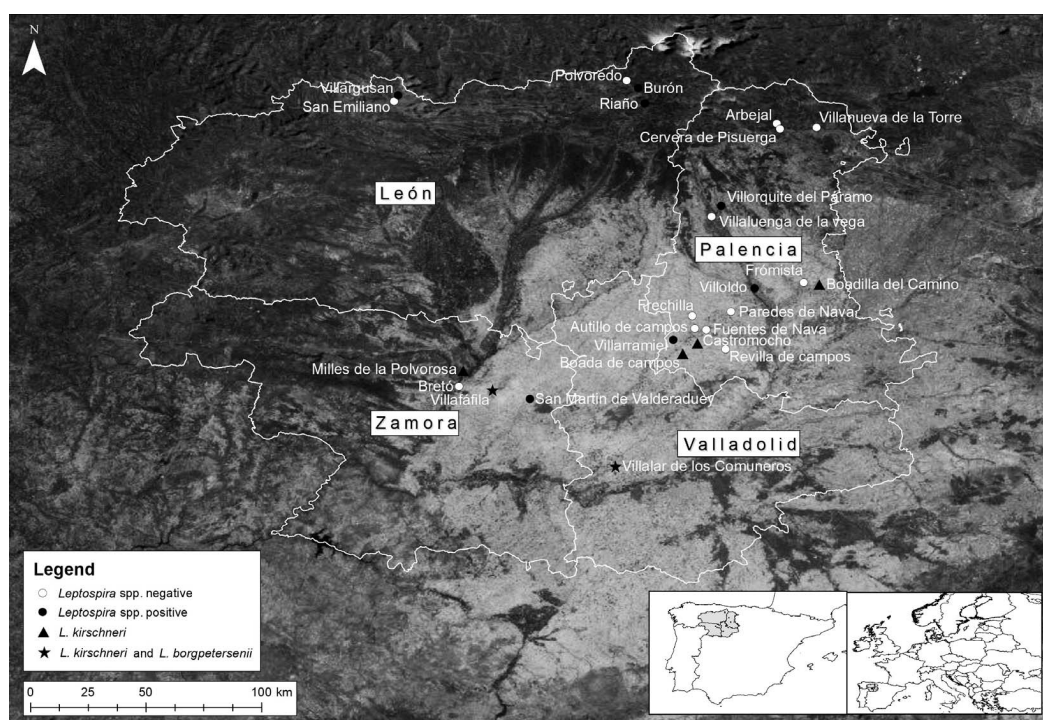
FIGURE 1.   Map of northwestern Spain with the trapping sites for common voles (*Microtus arvalis*) and Lusitanian pine voles (*Microtus lusitanicus*) in provinces Leon, Palencia, Zamora, and Valladolid, with inserts of the localization of the trapping region in Spain and Europe. Black dots mark locations where voles tested positive for *Leptospira* spp. DNA; white dots indicate locations without *Leptospira* DNA detection in voles. Detection of *L. kirschneri* and of both *L. kirschneri* and *L. borgpetersenii* is indicated at the vole trapping sites by triangles and stars, respectively. *Leptospira* spp. DNA was detected by PCR targeting the *lipL32* gene; genomospecies identification used *secY* PCR and sequence determination. The map was generated using ArcMap 10.5.1 (ESRI 2011).

The trapping location (site) was included as a random factor, accounting for the spatial design of the study. To account for missing values in the explanatory variables, we removed all rows with at least one missing value from the dataset (pruning). The loss of information was high (33%, 195 of 599 rows); thus, models were fitted also with datasets in which only the respective rows with missing values in the explanatory variable considered were excluded (not pruning). The results of both approaches were qualitatively similar (data not shown). Here, we only present the results of the analyses performed with the dataset without pruning. Because some explanatory variable values were not available, we did not perform Akaike information criterion (AIC)-based model selection because comparison across different models is only meaningful when fitted with the same dataset. Therefore, stepwise-backward and -forward model selection was used to determine the final regression models and to obtain a single minimum adequate model in which all variables

were $P<0.10$ (Crawley 2007). All analyses were performed in R software (version 3.6.1; R Development Core Team 2019) using the package lme4 (Bates et al. 2014).

RESULTS

Screening of 580 common voles and six Lusitanian pine voles (*Microtus lusitanicus*) with *lipL32* PCR allowed us to detect pathogenic *Leptospira* spp. in 46 common voles (7.9%; 95% confidence interval [CI], 5.9–10.4) and two Lusitanian pine voles (33.3%; 95% CI, 4.3–77.7; Supplementary Material Table S1). Infected voles were detected at 13 of 26 sites, in all four provinces (Fig. 1). In general, the DNA detection rate varied between the years (lowest in 2012 with 4.2%; 95% CI, 2.3–7.0; highest in 2014 with

TABLE 1.   Results of univariate binomial generalized linear mixed-effects models for the individual infection of common voles (*Microtus arvalis*) with *Leptospira* spp. collected in the provinces of Leon, Palencia, Zamora, and Valladolid, northwestern Spain, 2011 to 2014.

Variables tested in univariable models	Explanatory variables			
	Estimate	SE	Z value	*P* value[a]
Rainfall	0.014026	0.005855	2.396	**0.0166**
Weight	0.05496	0.01365	4.026	**0.000057**
Maximum temperature	0.06247	0.03762	1.660	0.0968
Relative air humidity	−0.02311	0.01775	−1.302	0.193
Phase population cycle (reference level: peak phase)	−0.9742	0.4766	−2.044	**0.0409**
Sex, male (reference level: female)	0.5347	0.3145	1.700	0.0891
Distance to water point log (water point meter)	0.0807	0.1220	0.662	0.508
Age class (reference level: adult)				
Juvenile	−1.5978	0.7571	−2.110	**0.0348**
Subadult	−1.1088	0.6275	−1.767	0.0772
Year (reference level: 2011)				
2012	−1.0183	1.3423	−0.759	0.4481
2013	0.2543	1.7659	0.144	0.8855
2014	0.2267	1.4111	0.161	0.8724

[a] Significant values are highlighted in bold (*P*<0.05).

13.1%; 95% CI, 9.2–18.3) and province (highest in León: 23.8%; 95% CI, 8.2–47.2; lowest in Valladolid: 5.1%; 95% CI, 0.6–17.3; Supplementary Material Table S1). The highest DNA detection rate at sites with at least 10 voles sampled was found in Palencia, Boada de Campos, and the average for the entire observation period was 16.4% (95% CI, 10.5–24.0) and, in 2014, was 20% (95% CI, 12.7–29.2). Typing by *secY* PCR revealed two *Leptospira* species in the common vole: *Leptospira kirschneri* (n=24) and *L. borgpetersenii* (n=2). All *L. kirschneri* samples selected were identified as ST 110 strains by MLST (Supplementary Material Table S1); MLST for *L. borgpetersenii* failed.

Results of the univariate analyses showed that the prevalence of *Leptospira* spp. was significantly associated with common vole weight, age class, rainfall, and phase of the population cycle (Table 1). Weight and age class had significant effects on *Leptospira* spp. prevalence when they were included separately in univariate GLMMs: adults had significantly higher prevalence than juveniles had (*P*=0.03) but not significantly higher prevalence than subadults had (*P*=0.07); weight effect was also significant (*P*=0.000057; see Table 1). Howev-

er, when both variables were included together into models, only weight was significant (*P*=0.000981; other *P*>0.05). The variable "phase of the population cycle" was significant in the model when included alone (*P*=0.04), but when it was included with other variables, the model did not converge. The best minimum adequate model showed positive effects of vole weight and rainfall (*P*<0.05; Table 2). The *P* value of maximum temperature (*P*=0.08) was slightly above the chosen significance (Table 2). Results of this model showed that, for each one-unit increase in weight, we expect about 6% increase in the probability of the common vole being infected (i.e., *P*=*lipL32*–PCR positive; Fig. 2). The model predicts that, maintaining a constant maximum air temperature at its mean value (27 C), the probability of infection of common voles with *Leptospira* spp. was very high (0.70–0.90) for weights >40 g and high amount of accumulated rainfall (250 mm) during the 90 d before capture (Fig. 2). We tested two- and three-way interactions of all the variables that were statistically significant in univariate GLMMs (see Table 1). In addition, interactions between each of these significant variables with the rest of the

TABLE 2.   The minimum adequate generalized linear mixed-effects model for the probability of individual infection of common voles (*Microtus arvalis*) with *Leptospira* spp. collected in northwestern Spain during 2011 to 2014. The vole trapping site was used as a random factor.

Variables tested in multivariable model	Explanatory variables			
	Estimate	SE	Z value	*P* value[a]
Intercept	7.250217	1.395408	5.196	**2.04e^{-07}*****
Weight	0.059013	0.013359	4.417	**9.99e^{-06}*****
Maximum temperature	0.069124	0.039647	1.744	0.0812
Rainfall	0.015472	0.006141	2.520	**0.0117***

[a] Significant values are highlighted in bold (*P*<0.05).

nonsignificant variables were tested and were found to be nonsignificant (data not shown).

For 384 common vole and four Lusitanian pine vole samples, the β-actin RT-PCR (internal control) was positive. However, the

FIGURE 2.   Probability of common vole (*Microtus arvalis*) being *lipL32*-PCR–positive (i.e., probability of vole being infected) as a function of vole weight and different amounts of accumulated rainfall during the 90 d before capture, for voles captured 2011–2014 in provinces Leon, Palencia, Zamora, and Valladolid, northwestern Spain. Points are observed values. Lines are predictions of the best multivariable generalized linear mixed-effects model (GLMM) for the probability of common vole being *lipL32*-PCR–positive at specific amounts of rainfall (mean=58 mm; maximum=250 mm), which was calculated as follows:

$$p = \frac{\exp(\beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k)}{1 + \exp(\beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k)}.$$

For the predictions, T_{max} was held constant at its mean value (27 C).

parallel hantavirus S- and L-segment RT-PCR assays had negative results for all samples (Supplementary Material Table S1).

DISCUSSION

Our molecular survey of voles from Spain for *Leptospira* spp. and TULV detected pathogenic *Leptospira* DNA in 46 of 580 (7.9%) common voles and in two of six (33%) Lusitanian pine voles. In previous studies in Spain, *Leptospira* DNA has been detected in various murine species, such as the wood mouse (*Apodemus sylvaticus*), the black rat (*Rattus rattus*), the house mouse (*Mus musculus*), and shrew species with a rate of 8% to 13% (Arent et al. 2017; Millan et al. 2018). Investigations in other European countries indicated DNA detection rates of 6.6% to 30% in common voles (Mayer-Scholl et al. 2014; Schmidt et al. 2014; Fischer et al. 2018; Kurucz et al. 2018; Blagojevic et al. 2019). Differences in the DNA detection rates might be due to different environmental conditions in Spain compared with those in central and southeastern European countries, which may influence survival of *Leptospira* spp. in the environment, transmission within the reservoir vole populations, and dynamics of vole populations and, thereby, the probability of pathogen transmission.

Our almost-exclusive detection of *L. kirschneri* and ST110 in common voles in Spain is in line with surveys in Germany (Mayer-Scholl et al. 2014; Fischer et al. 2018) and Austria (Jeske et al. 2021). The additional detection of *L. borgpetersenii* in our study may imply a
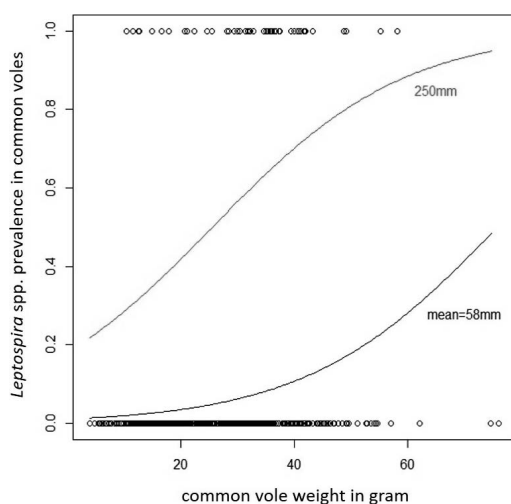
spillover from another rodent or insectivore species, as reported previously (Mayer-Scholl et al. 2014). Wood mice were reported as a carrier of *L. borgpetersenii* in Spain (Millan et al. 2018) and were also trapped in the same areas in which the *L. borgpetersenii*–positive common voles were detected in our study (data not shown). Field voles (*Microtus agrestis*) were found in Germany to be exclusively infected by *L. kirschneri* ST110, whereas bank voles (*Myodes glareolus*) and yellow-necked mice (*Apodemus flavicollis*) were found to be infected not only by *L. kirschneri* ST110 but also by *L. borgpetersenii* and *L. interrogans*, suggesting potential spillover infections among rodent species in grassland, agricultural, and forest habitats (Fischer et al. 2018).

The discrepancies between the number of *lipL*32-positive and *secY*/MLST-positive animals are due to the lower sensitivity of the *secY*-PCR in comparison to the *lipL*32 screening PCR and the lower sensitivity of the MLST (based on seven individual PCRs). Because both the *secY*-PCR–based genomospecies identification and MLST analysis rely on sequencing, which needs larger amounts of amplified DNA, the sensitivity of the methods is lower than that of the *lipL*32 PCR. This has been observed previously (Obiegala et al. 2016; Fischer et al. 2018).

Weight was strongly and positively correlated to *Leptospira* DNA detection rate in common voles, in line with previous studies (Cortez et al. 2018; Fischer et al. 2018). This is also seen in our study in the significantly lower infection rates in juvenile voles compared with adult voles. This relationship has also been observed in other rodent species, such as rats (Krøjgaard et al. 2009; Costa et al. 2014; Heuser et al. 2017), and explained as a consequence of a persistent infection (Benacer et al. 2016; Heuser et al. 2017; Minter et al. 2017).

Sex has been controversially discussed as a further demographic factor that influences the *Leptospira* prevalence (Benacer et al. 2016; Cortez et al. 2018). This potential influence in males might be explained by the immunosuppressive effect of androgens or by behaviors (aggressiveness, dispersal, foraging) that increase exposure to pathogens. However, neither our study nor previous studies found a significant sex effect on *Leptospira* prevalence (Benacer et al. 2016, Cortez et al. 2018).

We found a significant, positive correlation for abundance or phase of population cycle of common voles and *Leptospira* prevalence in the univariate GLMM (P=0.049; Table 1). During the peak phase of the population cycle with high population density of voles, spread of *Leptospira* by direct transmission among reservoir hosts (Faine et al. 1999) is enhanced. Indirect transmission may also have an important role in vole reservoirs because climatic factors, such as rainfall and temperature, were significant in the GLMM. Once the bacteria are excreted into the environment, factors such as temperature, pH-value, ultraviolet (UV) light, and moisture affect survival of the organism and, thus, transmission. The highest *Leptospira* incidences are reported in regions with a mean annual temperature of 20 C (Jensen and Magnussen 2016) because these bacteria require warm conditions for survival outside the host (Thibeaux et al. 2018). The mean annual temperature in the investigated region in Spain is lower (Climate-Data.org 2021), suggesting low *Leptospira* survival, except for the summer, in which the maximum temperature observed in our study area in Spain is close to the temperature of 30 C, which is reported to be optimal for *Leptospira* survival outside the host (Andre-Fontaine et al. 2015).

Soil humidity is also an important factor for the survival of *Leptospira* spp. outside the host (Schneider et al. 2018; Thibeaux et al. 2018) and, therefore, for its prevalence in rodents (Morand et al. 2019). This can be mediated either by rainfall, which was positively correlated to *Leptospira* prevalence in our study, or by close water bodies, such as rivers and irrigation ditches (Ganoza et al. 2006; Morand et al. 2019), for which, we found no indication. In addition, we measured relative air humidity as a proxy, which was, however, not a significant factor in this study (Table 1; P=0.193). Heavy rainfall and flooding have been reported in several studies to

have a positive effect on *Leptospira* spp. prevalence in rodents (Perez et al. 2011; Ivanova et al. 2012; Mason et al. 2016; Cortez et al. 2018).

In our study, no TULV or related hantavirus RNA was detected in any of the voles investigated, although both a highly sensitive conventional and a broad-spectrum RT-rtPCR were used. This is in strong contrast to the TULV RNA detection rates of 11.8% to 40.8% reported for different regions in Europe (Scharninghausen et al. 2002; Schmidt et al. 2014, 2016; Maas et al. 2017; Kurucz et al. 2018; Saxenhofer et al. 2019). A possible reason for these negative results might be the long-term isolation of common voles on the Iberian Peninsula (Heckel et al. 2005; Fischer et al. 2014; Saxenhofer et al. 2017; García et al. 2020).

In conclusion, *Leptospira* spp. are important common vole–associated zoonotic pathogens in Spain. The role of Lusitanian pine voles as a potential reservoir of leptospires needs further attention because the number of such voles we sampled was very low. Additionally, the role of interspecies transmission of *Leptospira* spp. from the main rodent reservoir to other rodents needs further evaluation. The molecular typing approach requires further improvements because the sensitivity of the *secY* PCR and MLST seems to be only moderate. The risk of *Leptospira* infection for humans may increase after rainfall during population explosions of common voles. For evaluation of human infection risk, harmonized approaches are needed in a pan-European rodent-monitoring approach (Sonnenburg et al. 2017). These investigations should incorporate analysis of environmental conditions, such as rainfall and temperature, and soil features, including moisture. Finally, additional efforts are needed to clarify whether leptospirosis is an underreported disease. The lack of TULV in voles from Spain might be explained by the evolutionary history of the isolated common vole population. Future investigations should also investigate whether the common vole in Spain acts as reservoir of additional (potential) zoonotic pathogens, such as cowpox virus (Prkno et al. 2017), hepevirus (Ryll et al. 2019), tick-borne encephalitis virus (Achazi et al. 2011), *Coxiella burnetii* (Literak 1995), *Bartonella* spp. (Rodriguez-Pastor et al. 2018), and *Francisella tularensis* (Rodriguez-Pastor et al. 2017).

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-20-00109.

LITERATURE CITED

Achazi K, Ruzek D, Donoso-Mantke O, Schlegel M, Ali HS, Wenk M, Schmidt-Chanasit J, Ohlmeyer L, Ruhe F, Vor T, et al. 2011. Rodents as sentinels for the prevalence of tick-borne encephalitis virus. *Vector Borne Zoonotic Dis* 11:641–647.

Andre-Fontaine G, Aviat F, Thorin C. 2015. Waterborne leptospirosis: Survival and preservation of the virulence of pathogenic *Leptospira* spp. in fresh water. *Curr Microbiol* 71:136–142.

Arent ZJ, Gilmore C, San-Miguel Ayanz JM, Neyra LQ, Garcia-Peña FJ. 2017. Molecular epidemiology of *Leptospira* serogroup Pomona infections among wild and domestic animals in Spain. *Ecohealth* 14:48–57.

Bates D, Mächler M, Bolker B, Walker S. 2014. Fitting linear mixed-effects models using lme4. *J. Stat. Softw* 67:1–48.

Benacer D, Mohd-Zain SN, Sim SZ, Mohd Khalid MK, Galloway RL, Souris M, Thong KL. 2016. Determination of *Leptospira borgpetersenii* serovar Javanica and *Leptospira interrogans* serovar Bataviae as the

persistent *Leptospira* serovars circulating in the urban rat populations in Peninsular Malaysia. *Parasit Vectors* 9:117.

Blagojevic J, Sekler M, Rajicic M, Peijic I, Budinski I, Jovanovic VM, Adnadevic T, Vidanovic D, Matovic K, Vujosevic M. 2019. The prevalence of pathogenic forms of *Leptospira* in natural populations of small wild mammals in Serbia. *Acta Vet Hung* 67:338–346.

Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthieka-nun V, Bailey MS, Holden MT, Zhang C, Jiang X, Koizumu N, Taylor K, et al. 2013. A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. *PLoS Negl Trop Dis* 7: e1954.

Climate-Data.org. 2021. *Spain climate/Spain weather by month*. https://en.climate-data.org/europe/spain-5/. Accessed January 2021.

Cortez V, Canal E, Dupont-Turkowsky JC, Quevedo T, Albujar C, Chang TC, Salmon-Mulanovich G, Guezala-Villavicencio MC, Simons MP, Margolis E, et al. 2018. Identification of *Leptospira* and *Bartonella* among rodents collected across a habitat disturbance gradient along the inter-oceanic highway in the southern Amazon Basin of Peru. *PLoS One* 13: e0205068.

Costa F, Porter FH, Rodrigues G, Farias H, de Faria MT, Wunder EA, Osikowicz LM, Kosoy MY, Reis MG, Ko AI, et al. 2014. Infections by *Leptospira interrogans*, Seoul virus, and *Bartonella* spp. among Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil. *Vector Borne Zoonotic Dis* 14:33–40.

Crawley MJ. 2007. *The R book*. John Wiley & Sons, Chichester, UK.

Desai S, Van Treek U, Lierz M, Espelage W, Zota L, Darbu A, Czerwinski M, Sadkowska-Todys M, Avdicova M, et al. 2009. Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. *Clin Infect Dis* 48:691–697.

Elliott RM. 1990. Molecular biology of the Bunyaviridae. *J Gen Virol* 71:501–522.

ESRI. 2017. ArcGIS version 10.5.1. Environmental Systems Research Institute, Redlands, California.

Faine S, Adler B, Bolin C, Perolat P. 1999. *Leptospira and leptospirosis*. MediSci, Melbourne, Australia.

Fávero JF, de Araújo HL, Lilenbaum W, Machado G, Tonin AA, Baldissera MD, Stefani LM, Da Silva AS. 2017. Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. *Microb Pathog*, 107:149–154.

Fischer MC, Foll M, Heckel G, Excoffier L. 2014. Continental-scale footprint of balancing and positive selection in a small rodent (*Microtus arvalis*). *PLoS One* 9:e112332.

Fischer S, Mayer-Scholl A, Imholt C, Spierling NG, Heuser E, Schmidt S, Reil D, Rosenfeld UM, Jacob J, Nöckler K, et al. 2018. *Leptospira* genomospecies and sequence type prevalence in small mammal populations in Germany. *Vector Borne Zoonotic Dis* 18:188–199.

Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, Segura ER, Gilman RH, Gotuzzo E, Vinetz JM. 2006. Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic *Leptospira*. *PLoS Med* 3:e308.

García JT, Domínguez-Villaseñor J, Alda F, Calero-Riestra M, Pérez Olea P, Fargallo JA, Martínez-Padilla J, Herranz J, Oñate JJ, Santamaría A. 2020. A complex scenario of glacial survival in Mediterranean and continental refugia of a temperate continental vole species (*Microtus arvalis*) in Europe. *J Zool Syst Evol Res*. 58:459–474.

Heckel G, Burri R, Fink S, Desmet JF, Excoffier L. 2005. Genetic structure and colonization processes in European populations of the common vole, *Microtus arvalis*. *Evolution* 59:2231–2242.

Heuser E, Fischer S, Ryll R, Mayer-Scholl A, Hoffmann D, Spahr C, Imholt C, Alfa DM, Frohlich A, Luschow D, et al. 2017. Survey for zoonotic pathogens in Norway rat populations from Europe. *Pest Manag Sci* 73:341–348.

Hiltbrunner M, Heckel G. 2020. Assessing genome-wide diversity in European hantaviruses through sequence capture from natural host samples. *Viruses* 12:749.

Ivanova S, Herbreteau V, Blasdell K, Chaval Y, Buchy P, Guillard B, Morand S. 2012. *Leptospira* and rodents in Cambodia: environmental determinants of infection. *Am J Trop Med Hyg* 86:1032–1038.

Jacob J, Tkadlec E. 2010. Rodent outbreaks in Europe: Dynamics and damage. In: *Rodent outbreaks: Ecology and impacts*. Singleton GR, Belmain SR, Brown PR, Hardy B, editors. International Rice Research Institute, Metro Manila, Philippines, pp. 207–223.

Jensen PM, Magnussen E. 2016. Is it too cold for *Leptospira interrogans* transmission on the Faroese Islands? *Infect Dis (Lond)* 48:156–160.

Jeske K, Herzig-Straschil B, Răileanu C, Kunec D, Tauchmann O, Emirhar D, Schmidt S, Trimpert J, Silaghi C, Heckel G, et al. 2021. Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria. *Transbound Emerg Dis*: In press.

Karpagam KB, Ganesh B. 2020. Leptospirosis: A neglected tropical zoonotic infection of public health importance-an updated review. *Eur J Clin Microbiol Infect Dis*. 39:835–846.

Klempa B, Meisel H, Räth S, Bartel J, Ulrich R, Krüger DH. 2003. Occurrence of renal and pulmonary syndrome in a region of northeast Germany where Tula hantavirus circulates. *J Clin Microbiol* 41:4894–4897.

Krøjgaard LH, Villumsen S, Markussen MD, Jensen JS, Leirs H, Heiberg AC. 2009. High prevalence of *Leptospira* spp. in sewer rats (*Rattus norvegicus*). *Epidemiol Infect* 137:1586–1592.

Kukkonen SK, Vaheri A, Plyusnin A. 1998. Completion of the Tula hantavirus genome sequence: properties of the L segment and heterogeneity found in the 3′ termini of S and L genome RNAs. *J Gen Virol* 79: 2615–2622.

Kurucz K, Madai M, Bali D, Hederics D, Horavth G, Kemenesi G, Jakab F. 2018. Parallel survey of two widespread renal syndrome-causing zoonoses: *Leptospira* spp. and hantavirus in urban environment, Hungary. *Vector Borne Zoonotic Dis* 18:200–205.

Literak I. 1995. [Occurrence of *Coxiella burnetti* antibodies in cattle, sheep and small terrestrial mammals in the western region of Bohemia]. *Vet Med (Praha)* 40:77–80. Czech.

Lledó L, Serrano JL, Giménez-Pardo C, Gegúndez I. 2020. Wild red foxes (*Vulpes vulpes*) as sentinels of rodent-borne hantavirus and lymphocytic choriomeningitis virus in the province of Soria, northern Spain. *J Wildl Dis* 56:658–661.

Luque-Larena JJ, Mougeot F, Roig DV, Lambin X, Rodriguez-Pastor R, Rodriguez-Valin E, Anda P, Escudero R. 2015. Tularemia outbreaks and common vole (*Microtus arvalis*) irruptive population dynamics in northwestern Spain, 1997–2014. *Vector Borne Zoonotic Dis* 15:568–570.

Maas M, de Vries A, van Roon A, Takumi K, van der Giessen J, Rockx B. 2017. High Prevalence of Tula hantavirus in common voles in the Netherlands. *Vector Borne Zoonotic Dis* 17:200–205.

Marquez A, Ulivieri T, Benoit E, Kodjo A, Lattard V. 2019. House mice as a real sanitary threat of human and animal leptospirosis: Proposal for integrated management. *Biomed Res Int* 2019:3794876.

Mason MR, Encina C, Sreevatsan S, Munoz-Zanzi C. 2016. Distribution and diversity of pathogenic *Leptospira* species in peri-domestic surface waters from south central Chile. *PLoS Negl Trop Dis* 10:e0004895.

Mayer-Scholl A, Draeger A, Luge E, Ulrich RG, Nöckler K. 2011. Comparison of two PCR systems for the rapid detection of *Leptospira* spp. from kidney tissue. *Curr Microbiol* 62:1104–1106.

Mayer-Scholl A, Hammerl JA, Schmidt S, Ulrich RG, Pfeffer M, Woll D, Scholz HC, Thomas A, Nöckler K. 2014. *Leptospira* spp. in rodents and shrews in Germany. *Int J Environ Res Public Health* 11:7562–7574.

Millan J, Cevidanes A, Chirife AD, Cendela MG, Leon-Vizcaino L. 2018. Risk factors of *Leptospira* infection in Mediterranean periurban micromammals. *Zoonoses Public Health* 65:e79–e85.

Minter A, Diggle PJ, Costa F, Childs J, Ko AI, Begon M. 2017. Evidence of multiple intraspecific transmission routes for *Leptospira* acquisition in Norway rats (*Rattus norvegicus*). *Epidemiol Infect* 145:3438–3448.

Morand S, Blasdell K, Bordes F, Buchy P, Carcy B, Chaisiri K, Chaval Y, Claude J, Cosson JF, Desquesnes M, et al. 2019. Changing landscapes of Southeast Asia and rodent-borne diseases: decreased diversity but increased transmission risks. *Ecol Appl* 29:e01886.

Morris, P. 1972. A review of mammalian age determination methods. *Mamm Rev* 2:69–104.

Mwachui M A, Crump L, Hartskeerl R, Zinsstag J, Hattendorf J. 2015. Environmental and behavioural determinants of leptospirosis transmission: a systematic review. *PLoS Negl Trop Dis* 9:e0003843.

Nikolic V, Stajkovic N, Stamenkovic G, Cekanac R, Marusic P, Siljic M, Gligic A, Stanojevic M. 2014. Evidence of recombination in Tula virus strains from Serbia. *Infect Genet Evol* 21:472–478.

Obiegala A, Woll D, Karnath C, Silaghi C, Schex S, Essbauer S, Pfeffer M. 2016. Prevalence and genotype allocation of pathogenic *Leptospira* species in small mammals from various habitat types in Germany. *PLoS Negl Trop Dis* 10:e0004501.

Perez J, Brescia F, Becam J, Mauron C, Goarant C. 2011. Rodent abundance dynamics and leptospirosis carriage in an area of hyper-endemicity in New Caledonia. *PLoS Negl Trop Dis* 5:e1361.

Prkno A, Hoffmann D, Goerigk D, Kaiser M, van Maanen ACF, Jeske K, Jenckel M, Pfaff F, Vahlenkamp TW, Beer M, et al. 2017. Epidemiological investigations of four cowpox virus outbreaks in alpaca herds. *Viruses* 9:344.

R Core Team. 2019. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed February 2020.

Reynes JM, Carli D, Boukezia N, Debruyne M, Herti S. 2015. Tula hantavirus infection in a hospitalised patient, France, June 2015. *Euro Surveill* 20:50.

Rodriguez-Pastor R, Escudero R, Lambin X, Vidal MD, Gil H, Jado I, Rodriguez-Vargas M, Luque-Larena JJ, Mougeot F. 2018. Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: Occurrence and dynamics. *Parasitology* 146:389–398.

Rodriguez-Pastor R, Escudero R, Vidal D, Mougeot F, Arroyo B, Lambin X, Vila-Coro AM, Rodriguez-Moreno I, Anda P, Luque-Larena JJ. 2017. Density-dependent prevalence of *Francisella tularensis* in fluctuating vole populations, northwestern Spain. *Emerg Infect Dis* 23:1377–1379.

Ryll R, Heckel G, Corman VM, Drexler JF, Ulrich RG. 2019. Genomic and spatial variability of a European common vole hepevirus. *Arch Virol* 164:2671–2682.

Sanfeliu I, Nogueras MM, Gegúndez MI, Segura F, Lledó L, Font B, Saz JV. 2011. Seroepidemiological survey of hantavirus infection in healthy people in Vallès Occidental, Barcelona. *Vector Borne Zoonotic Dis* 11:697–700.

Saxenhofer M, Schmidt S, Ulrich RG, Heckel G. 2019. Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus. *PLoS Biol* 17:e3000142.

Saxenhofer M, Weber de Melo V, Ulrich RG, Heckel G. 2017. Revised time scales of RNA virus evolution based on spatial information. *Proc Biol Sci* 284:20170857.

Scharninghausen JJ, Pfeffer M, Meyer H, Davis DS, Honeycutt RL, Faulde M. 2002. Genetic evidence for Tula virus in *Microtus arvalis* and *Microtus agrestis* populations in Croatia. *Vector Borne Zoonotic Dis* 2:19–27.

Schlegel M, Ali HS, Stieger N, Groschup MH, Wolf R, Ulrich RG. 2012a. Molecular identification of small mammal species using novel cytochrome B gene-derived degenerated primers. *Biochem Genet* 50: 440–447.

Schlegel M, Kindler E, Essbauer SS, Wolf R, Thiel J, Groschup MH, Heckel G, Oehme RM, Ulrich RG. 2012b. Tula virus infections in the Eurasian water vole in Central Europe. *Vector Borne Zoonotic Dis* 12:503–513.

Schmidt S, Essbauer SS, Mayer-Scholl A, Poppert S, Schmidt-Chanasit J, Klempa B, Henning K, Schares G, Groschup MH, Spitzenberger F, et al. 2014. Multiple infections of rodents with zoonotic pathogens in Austria. *Vector Borne Zoonotic Dis* 14:467–475.

Schmidt S, Saxenhofer M, Drewes S, Schlegel M, Wanka KM, Frank R, Klimpel S, von Blanckenhagen F, Maaz D, Herden C, et al. 2016. High genetic structuring of Tula hantavirus. *Arch Virol* 161:1135–1149.

Schneider AG, Casanovas-Massana A, Hacker KP, Wunder EA, Begon M Jr, Reis MG, Childs JE, Costa F, Lindow JC, Ko AI. 2018. Quantification of pathogenic *Leptospira* in the soils of a Brazilian urban slum. *PLoS Negl Trop Dis* 12:e0006415.

Sibold C, Meisel H, Krüger DH, Labuda M, Lysy J, Kozuch O, Pejcoch M, Vaheri A, Plyusnin A. 1999. Recombination in Tula hantavirus evolution: Analysis of genetic lineages from Slovakia. *J Virol* 73:667–675.

Sonnenburg J, Ryser-Degiorgis MP, Kuiken T, Ferroglio E, Ulrich RG, Conraths FJ, Gortazar C, Staubach C. 2017. Harmonizing methods for wildlife abundance estimation and pathogen detection in Europe—A questionnaire survey on three selected host-pathogen combinations. *BMC Vet Res* 13:53.

Thibeaux R, Girault D, Bierque E, Soupe-Gilbert ME, Rettinger A, Douyere A, Meyer M, Iraola G, Picardeau M, Goarant C. 2018. Biodiversity of environmental *Leptospira*: Improving identification and revisiting the diagnosis. *Front Microbiol* 9:816.

Victoria B, Ahmed A, Zuerner RL, Ahmed N, Bulach DM, Quinteiro J, Hartskeerl RA. 2008. Conservation of the *S10-spc-α* locus within otherwise highly plastic genomes provides phylogenetic insight into the genus *Leptospira*. *PLoS One* 3:e2752.

Wakeley PR, Johnson N, McElhinney LM, Marston D, Sawyer J, Fooks AR. 2005. Development of a real-time, TaqMan reverse transcription-PCR assay for detection and differentiation of Lyssavirus genotypes 1, 5, and 6. *J Clin Microbiol* 43:2786–2792.

Zelená H, Mrázek J, Kuhn T. 2013. Tula hantavirus infection in immunocompromised host, Czech Republic. *Emerg Infect Dis* 19:1873–1875.

Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-20-00109: Kathrin Jeske, Duygu Emirhar, Jesús T. García, David González-Barrio, Pedro P. Olea, Francisco Ruiz Fons, Jana Schulz, Anne Mayer-Scholl, Gerald Heckel, and Rainer G. Ulrich. Frequent *Leptospira* spp. detection but absence of Tula orthohantavirus in *Microtus* spp. voles, northwestern Spain.

MATERIAL AND METHODS

Between 2011 and 2014, voles were trapped at 26 sampling sites within the western part of the Duero river basin, northwestern Spain (Fig. 1 and Table S1). These sites were selected as they cover a significant part of the area newly colonized by common voles (*Microtus arvalis*) during the last 50 years (Garcia et al. 2020). The region was traditionally dominated by extensive cultivation of cereal crops (mainly wheat and barley), but the recent introduction of different irrigation crops (mainly alfalfa, corn and some winter cereals) has created a landscape composed of discrete irrigated and non-irrigated areas in which different farming methods are mixed. Since our main objective was to maximize the collection of samples to guarantee a sufficient number of captures at each site, we used different trapping protocols in this study: some sites were sampled by eight 7 x 7 trapping grids (392 traps/site), others by 3-10 trap lines, consisting of 12 traps with 5 m between traps in each line, and some other sites by distributing traps in 15–80 capture points per study site, with 10 traps per point (150–800 traps per study site). In all cases, we used LFATDG Sherman Live Traps (7.62 cm × 8.89 cm × 22.86 cm, H. B. Sherman Traps, Inc., Tallahassee, Florida, USA) without or with bait (carrot or apple slices). We tried to ensure that trap locations encompassed the diversity of habitats at each site (e.g., crop fields, fallows, field margins, boundaries of roads and rural tracks, ditches). To maximize trapping effectiveness, we placed traps on active burrow systems whenever present, on inactive ones or randomly within the fields and margins when no burrows were found at all. Traps were

1

98

open for 24 h, or until captures reached a minimum of at least 10 individuals per site, which usually took no more than 48 h. We georeferenced all the capture points in the field with a GPS device. Captures were made in different months throughout the study period, covering all seasons of the year. Individuals were captured and sedated with an intramuscular injection of a solution containing ketamine (10 mg/kg; Imalgene; Boehringer Ingelheim, Barcelona, Spain) and medetomidine (1 mg/kg; Medetor; CP-Pharma Handelsges., Burgdorf, Germany) and thereafter humanely euthanised by cervical dislocation.

Carcasses of trapped animals were transported refrigerated to our labs where they were weighed and age class (juvenile (less than 14.5 g), subadult (14.5 up to 19.5 g), adult (more than 19.5 g; Morris 1972), sex and different biometric measurements were recorded. A detailed necropsy was performed under biosafety 2 containment in cabinets, and tissue samples were collected and preserved frozen at -20 C. For each animal, trapping date and site, and species were recorded. Additionally, the phase of the population cycle, as well as the distance to the nearest water point (in m) were recorded (see Table S2). Weather data were obtained during the 90 d prior to rodent capture (Agencia Estatal de Meteorología 2020, InfoRiego 2020; see Table S2).

LITERATURE CITED

Agencia Estatal de Meteorología. 2020. https://opendata.aemet.es/centrodedescargas/productosAEMET?. Accessed May 2020.

García JT, Domínguez-Villaseñor J, Alda F, Calero-Riestra M, Pérez Olea P, Fargallo JA, Martínez-Padilla J, Herranz J, Oñate JJ, Santamaría A. 2020. A complex scenario of glacial survival in Mediterranean and continental refugia of a temperate continental vole species (*Microtus arvalis*) in Europe. *J. Zool. Syst. Evol. Res.* 58: 459-474.

2

InfoRiego. 2020. http://www.inforiego.org/opencms/opencms. Accessed May 2020.

Jareño D. 2014. *Las plagas de topillo campesino (Microtus arvalis) en Castilla y León: efectos del clima, los cambios en el uso del suelo, e impacto sobre el ecosistema global*. Ciudad Real, Spain, Universidad de Castilla-La Mancha, doctoral thesis

Luque-Larena, JJ, Mougeot F, Viñuela J, Jareño D, Arroyo L, Lambin X, Arroyo, B. 2013. Recent large-scale range expansion and eruption of common vole (*Microtus arvalis*) outbreaks in NW Spain. *Basic Appl Ecol.* 14: 432-441

Mougeot F, Lambin X, Rodríguez-Pastor R, Romairone J, Luque-Larena JJ. 2019. Numerical response of a mammalian specialist predator to multiple prey dynamics in Mediterranean farmlands. *Ecology* 100: Article e02776

Morris P. 1972. A review of mammalian age determination methods. *Mamm Rev* 2:69-104

Paz A, Jareño D, Arroyo L, Viñuela J, Arroyo B, Mougeot F, Luque-Larena JJ, Fargallo JA. 2013. Avian predators as a biological control system of common vole (*Microtus arvalis*) populations in north-western Spain: experimental set-up and preliminary results. *Pest Manag Sci* 69:444-450

Planillo A, Viñuela J, Malo JE, García JT, Acebes P, Santamaría A, Domínguez J, Olea PP. Local crop management and vegetation structure affect common vole abundance in agricultural field margins over the population cycle. *Agriculture, Ecosystems, and Environment* (submitted).

Santamaría AE, Olea PP, Viñuela J, García JT. 2019. Spatial and seasonal variation in occupation and abundance of common vole burrows in highly disturbed agricultural ecosystems. *Eur J Wildl Res* 65: Article 52

3

Table S1. Results of *Leptospira* spp. and Tula orthohantavirus detection depending on province, trapping location, year, and species for common voles (*Microtus arvalis*) and Lusitanian pine voles (*Microtus lusitanicus*) in northwestern Spain, 2011-2014. MLST, multi locus sequence typing; n.d., not determined; n.a., not applicable; RT-PCR, reverse-transcription PCR; RT-rtPCR, reverse-transcription real-time PCR.

Province	Site	Year	Species	Number of trapped voles	*Leptospira* spp. PCR positive/tested	Prevalence in %	95% confidence interval	*secY*-based genomospecies identification	MLST	Tula orthohantavirus RT-PCR or RT-rtPCR positive/tested
León	Burón	2012	*M. arvalis*	7	2/7	28.6	3.7 – 71	n.d.	n.a.	0/7
	Polvoredo	2012	*M. lusitanicus*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
	Riaño	2012	*M. lusitanicus*	3	2/3	66.7	9.4 - 99.2	n.d.	n.a.	0/3
	San Emiliano	2012	*M. arvalis*	6	0/6	0	0 - 45.9	n.a.	n.a.	0/0
	Villargusan	2012	*M. arvalis*	4	1/4	25.0	0.6 - 80.60	n.d.	n.a.	0/0
	Subtotal			21	5/21	23.8	8.2 - 47.2	n.d.	n.a.	0/11
Palencia	Arbejal	2012	*M. arvalis*	32	0/32	0	0 - 10.9	n.a.	n.a.	0/18
	Autillo de campos	2012	*M. arvalis*	2	0/2	0	0 - 84.2	n.a.	n.a.	0/2
	Boada de campos	2012	*M. arvalis*	26	0/26	0	0 - 13.2	n.a.	n.a.	0/19
		2013	*M. arvalis*	2	1/2	50	1.3 - 98.7	n.d.	n.a.	0/1
		2014	*M. arvalis*	102	20/100	20	12.7 - 29.2	13x *L. kirschneri*	2x 110	0/65
	Boadilla del Camino	2011	*M. arvalis*	5	2/5	40	5.3 - 85.3	1x *L. kirschneri*	1x 110	0/2
	Castromocho	2012	*M. arvalis*	29	5/29	17.2	5.9 - 35.8	3x *L. kirschneri*	1x 110	0/23
	Cervera de Pisuerga	2012	*M. arvalis*	5	0/5	0	0 - 52.2	n.a.	n.a.	0/3
	Frechilla	2012	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/0
	Frómista	2011	*M. arvalis*	9	0/9	0	0 - 33.6	n.a.	n.a.	0/4
		2012	*M. arvalis*	2	0/2	0	0 - 84.2	n.a.	n.a.	0/1
	Fuentes de Nava	2012	*M. arvalis*	11	0/7	0	0 – 41.0	n.a.	n.a.	0/8
	Paredes de Nava	2012	*M. arvalis*	42	0/42	0	0 - 8.4	n.a.	n.a.	0/21
	Revilla de campos	2012	*M. arvalis*	5	0/5	0	0 - 52.2	n.a.	n.a.	0/5
		2013	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
	Villaluenga de la vega	2012	*M. arvalis*	15	0/15	0	0 - 21.8	n.a.	n.a.	0/14
			M. lusitanicus	1	0/1	0	0 - 97.5	n.a.	n.a.	0/0
	Villanueva de la Torre	2012	*M. arvalis*	23	0/13	0	0 - 24.7	n.a.	n.a.	0/23
	Villarramiel	2014	*M. arvalis*	13	1/13	7.7	0.2 - 36.0	1x *L. kirschneri*	n.d.	0/13
	Villoldo	2012	*M. arvalis*	36	1/34	2.9	0.1 - 15.3	n.d.	n.a.	0/20
	Villorquite del	2012	*M. arvalis*	28	1/28	3.6	0.1 - 18.4	n.d.	n.a.	0/27
	Páramo		*M. lusitanicus*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/0
	Subtotal	391	31/383	8.1	5.6 - 11.3	n.d.			0/260	0/270

4

Table S1 (continued)

Province	Town	Year	Species	Number of trapped voles	*Leptospira* spp. PCR positive/ tested	Prevalence in %	95 confidence interval	*secY*-based genomospecies identification	MLST	Tula orthohantavirus RT-PCR and/ or RT-qPCR positive/ tested
Valladolid	Villalar de los Comuneros	2013	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
	Comuneros	2014	*M. arvalis*	38	2/38	5.3	0.64-17.8	1x *L. kirschneri* 1x *L. borgptersenii*	n.d.	0/24
	Subtotal			39	2/39	5.1	0.6 - 17.3	n.d.	n.a.	0/25
Zamora	Bretó	2012	*M. arvalis*	8	0/6	0	0 - 45.9	n.a.	n.a.	0/2
	Milles de la Polvorosa	2012	*M. arvalis*	19	1/16	6.3	0.2 - 30.2	1x *L. kirschneri*	1x 110	0/6
	San Martin de	2012	*M. arvalis*	19	1/19	5.3	0.1 - 26.0	n.d.	n.a.	0/15
	Valderaduey	2014	*M. arvalis*	11	0/11	0	0.0 - 28.5	n.a.	n.a.	0/7
	Villafáfila	2012	*M. arvalis*	16	0/16	0	0 – 20.6	n.a.	n.a.	0/13
		2013	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
		2014	*M. arvalis*	72	8/72	11.1	4.9 - 20.7	4x *L. kirschneri* 1x *L. borgpetersenii*	2x 110	0/49
	Subtotal			146	10/141	7.1	3.5 – 12.75	n.a.	n.a.	0/93
Total			*M. arvalis*	591	46/580	7.9	5.9 - 10.4	24x *L. kirschneri* 2x *L. borgptersenii*	6x 110	0/384
			M. lusitanicus	6	2/6	33.3	4.3 - 77.7	n.d.	n.a.	0/4
			Microtus spp.	597	48/586	8.2	6.1 - 10.7	24x *L. kirschneri* 2x *L. borgptersenii*	6x 110	0/388

102

5

Table S2. Explanatory variables that were analyzed using univariate and generalized linear mixed-effects model (GLMM) to investigate the prevalence of *Leptospira* spp. in individuals of the common vole (*Microtus arvalis*) trapped in northwestern Spain, 2011-2014.

Variable	Description of variables
Weight	in grams, to the nearest 0.1 gram
Age class	juvenile, subadult and adult
Sex	male, female
Distance to the next water body	used as a proxy for soil moisture, logarithm of distance in meter
Maximum temperature	in C, the single maximum temperature registered throughout the 90 days prior to capture (InfoRiego 2020, Agencia Estatal de Meteorología)
Rainfall	in mm, accumulated rainfall during the 90 days prior to capture (InfoRiego 2020, Agencia Estatal de Meteorología)
Relative humidity	in %, mean of the relative humidity registered per day in the 90 days prior to capture (InfoRiego 2020, Agencia Estatal de Meteorología)
Phases of the population cycle in which the common voles were at the capture date	high abundance (peak phase)* versus low abundance** (Luque-Larena et al. 2013, Paz et al., 2013, Jareño 2014, Mougeot et al. 2019, Santamaría et al. 2019). The different population cycle phases (increasing, decreasing, peak and crash or low abundance) were established over long temporal series of abundance data in the study area after Jareño (2014), Mougeot et al. (2019) and Planillo et al. (submitted).
Year	2012, 2013, and 2014

*27.7 vole captures per 100 traps; **0.23 vole captures per 100 traps

6

3.4 Paper IV

Jeske K, Jacob J, Drewes S, Pfeffer M, Heckel G, Ulrich RG, Imholt C. Hantavirus - *Leptospira* coinfections in small mammals from central Germany. Epidemiology and Infection (submitted)

Original Paper

Hantavirus–*Leptospira* coinfections in small mammals from central Germany

K. Jeske[1,2] (iD), J. Jacob[3] (iD), S. Drewes[1] (iD), M. Pfeffer[4] (iD), G. Heckel[5] (iD), R. G. Ulrich[1] (iD) and C. Imholt[3] (iD)

[1]Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany; [2]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany; [3]Vertebrate Research, Institute for Plant Protection in Horticulture and Forests, Julius Kühn-Institute, Toppheideweg 88, 48161 Münster, Germany; [4]Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, An den Tierkliniken 1, 04103 Leipzig, Germany and [5]Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

Abstract

European orthohantaviruses (Puumala orthohantavirus (PUUV); Dobrava-Belgrade orthohantavirus (DOBV), genotype Kurkino; Tula orthohantavirus (TULV)), and *Leptospira* spp. are small mammal-associated zoonotic pathogens that cause diseases with potentially similar symptoms in humans. We investigated the frequency of *Leptospira* spp. and hantavirus single and double infections in small mammals from 22 sites in Thuringia, central Germany, during 2017. TULV infections were detected at 18 of 22 sites (mean prevalence 13.8%, 93/674). PUUV infections were detected at four of 22 sites (mean prevalence 1.5%, 7/471), and respective PUUV sequences formed a novel phylogenetic clade, but DOBV infections were not detected at all. *Leptospira* infections were detected at 21 of 22 sites with the highest overall prevalence in field voles (*Microtus agrestis*) with 54.5% (6/11) and common voles (*Microtus arvalis*) with 30.3% (205/676). *Leptospira*–hantavirus coinfections were found in 6.6% (44/671) of common voles but only in two of 395 bank voles. TULV and *Leptospira* coinfection probability in common voles was driven by individual (age) and population-level factors. Coinfections seemed to be particularly associated with sites where *Leptospira* spp. prevalence exceeded 35%. Future investigations should evaluate public health consequences of this strong spatial clustering of coinfections.

Introduction

Coinfections of multiple pathogens can influence epidemiology and disease severity [1]. An understanding of ecological drivers of coinfections is important to improve a targeted public health response. Human infections by zoonotic orthohantaviruses and *Leptospira* spp. are (re-)emerging zoonoses that are almost indistinguishable in their clinical presentation [2] and can often be mistaken for each other.

Leptospira spp. are gram-negative bacteria of the class *Spirochaetes*, order *Leptospirales*, family *Leptospiraceae* and are 6–20 µm in size and 0.1 µm in diameter [3]. They can be divided into saprophytic, intermediate and pathogenic groups (including *L. kirschneri*, *L. borgpetersenii* and *L. interrogans*) [4]. Human infections can occur after contact with infected animals or indirectly through contact with contaminated water or soil. The disease course is in most cases asymptomatic or mild, but can progress in some cases after a febrile phase to multiple organ dysfunction [5]. Human incidences vary globally, with amplifying factors (tropical climate, standing water and low sanitation level) being notably absent at higher latitudes [6]. Rodents and shrews are considered as reservoir hosts for zoonotic *Leptospira* spp. with prevalences reaching 50% depending on species and season [4].

Hantaviruses, order *Bunyavirales*, family *Hantaviridae*, are enveloped viruses with a three segmented RNA genome of negative polarity [7]. Depending on the species, orthohantaviruses can cause haemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome. There is an estimated 150 000 cases of HFRS each year, with more than half occurring in China [8]. In Central Europe, Puumala orthohantavirus (PUUV) is the most important hantavirus as reflected by the large number of human cases, in particular during outbreak years. In Germany, the mean incidence is 0.87 per 100 000 inhabitants [9], but it reached 60 per 100 000 inhabitants in the outbreak year 2012 in the districts Göppingen and Heidenheim in Baden-Wuerttemberg [10]. Although the reservoir of PUUV, the bank vole (*Clethrionomys glareolus*), is distributed throughout Germany, PUUV is present only in the southern and western parts of the country [11]. The occurrence of Dobrava-Belgrade orthohantavirus (DOBV), genotype Kurkino, in Germany follows the geographical distribution of its reservoir, the striped field mouse (*Apodemus agrarius*) and is limited to north-eastern

CAMBRIDGE
UNIVERSITY PRESS

and eastern Germany [9, 12]. Finally, Tula orthohantavirus (TULV) is a broadly distributed orthohantavirus with the common vole (*Microtus arvalis*) as reservoir, but was also detected in other closely related species such as the field vole (*Microtus agrestis*), East European vole (*Microtus levis*) and water vole (*Arvicola amphibius*) [13]. TULV is generally considered to be of no (or low) pathogenicity, with only sporadic evidence of human infections [13, 14].

Coinfection with both pathogens has been confirmed in humans and rodents [15, 16] and in this study, we screened rodents and shrews from central Germany over the course of a year for pathogenic *Leptospira* spp., TULV, DOBV and PUUV and evaluated the frequency of dual hantavirus–*Leptospira* infections.

Material and methods

Trapping and dissection

Small mammals were trapped in spring, summer and fall 2017 at 22 sites in Thuringia, central Germany (Fig. 1). In central Germany, the distributional ranges of all abovementioned pathogens and their hosts probably overlap [4, 13, 17]. Each site consisted of perennial grassland as well as the adjacent grassland-forest ecotone. In each of these habitats small mammals were trapped with 36 snap traps (metal snap traps, Deufa, Neuburg, Germany) set in four rows with 10 m trap spacing. In the ecotone, two rows were set in the grassland section and two rows in the transition to the prevailing forest habitat. The trapping at site UH6 was discontinued after spring season due to logistic reasons. All procedures involving animals were conducted according to relevant legislation and by permission of the Thuringia state office of Consumer Protection (permit 22-2684-04-15-105/16). Small mammal carcasses were frozen at −20 °C until dissection. During dissection, small mammals were measured, weighed and sex was determined. To avoid contamination, sterile instruments for each individual were used. Lung and kidney tissue were collected and stored at −20 °C. If necessary, species and sex were determined by corresponding polymerase chain reaction (PCR) assays using kidney tissue-derived DNA as previously described [4, 18].

Leptospira spp. DNA screening

A pin-head-sized piece of kidney tissue was used for DNA extraction by Tissue DNA Kit according to the manual of the manufacturer (Roboklon, Berlin, Germany). DNA concentration was determined with Nanodrop ND-1000 (peqlab Biotechnologie GmbH, Erlangen, Germany). DNA samples were tested in the conventional *lipL32* PCR for the presence of pathogenic leptospires [4, 18]. Genomospecies identification of positive samples was done by *secY* PCR, dideoxy chain termination sequencing of PCR products with BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems™, Waltham, MA, USA) and sequence comparison to GenBank entries by nucleotide Basic Local Alignment Search Tool (BLASTn) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) [4].

Hantavirus screening by RT-PCR

RNA was extracted from a lentil-sized piece of lung tissue with QIAZOL reagent (QIAGEN, Hilden, Germany) and eluted in 100 µl DNase/RNase free water (Thermo Fisher Scientific, Schwerte, Germany) [13]. RNA concentration was measured with Nanodrop ND-1000. Reverse transcription-PCR (RT-PCR) was performed using SuperScript™ III One-Step RT-PCR with Platinum Taq-Kit (Invitrogen, Darmstadt, Germany). TULV/PUUV S segment RT-PCR screening used the primer pair PUUV342F and PUUV1102R [19]. DOBV RNA screening was based on RT-PCR using the S segment primer pair D113M and D955CM [20]. RT-PCR products of the expected size were directly sequenced using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems™).

Phylogenetic analysis

ClustalW multiple alignments of obtained nucleotide (nt)-sequences were constructed using BioEdit v7.2.5 [21]. The best fitting substitution model was determined by jModelTest v2.1.6 [22]. Phylogenetic trees were reconstructed according to maximum likelihood and Bayesian algorithms via FasttreeMP v2.1.10 and MrBayes v3.2.6 on CIPRES Science Gateway [23–25]. Subsequently, a consensus tree was established as bootstrap values ≥75 of the maximum likelihood tree were transferred to the Bayesian tree only if branches of both trees were consistent. Probabilities of node support of the Bayesian tree are given when the value was ≥95%.

Statistical analysis

To estimate key drivers of coinfections, a generalised linear mixed model was generated for *Microtus* spp. in grassland, where the individual coinfection status (binomial variable; TULV RNA positive and *Leptospira* spp. DNA positive) was the dependent variable. Individual demographic variables (sex, weight as a proxy for age) [26] as well as population level variables (TULV prevalence, *Leptospira* spp. prevalence, abundance (trap success as individuals per 100 trap nights), abundance in the previous season and season itself) were fixed factors. Site was incorporated as a random factor. The most appropriate model was determined using a multimodel inference approach. Using the *dredge* function from the *MuMIn*-package all possible combinations of fixed factors were ranked by their conditional Akaike information criterion (AIC). The best fitting models were defined as being within a ΔAIC of <2 of the best model (lowest AIC). Model coefficients were averaged using the *model.avg* function. We present the relative importance for each factor as the sum of Akaike weights in the best fitting models where the respective factor occurs as well as the 95% confidence interval (CI) for each factor. Here, a factor can be considered significant if the CIs do not include zero.

As trap success of *Microtus* spp. in the grassland/forest ecotone precluded a full model, a chi-square test was used to compare the overall prevalence in both habitats. CIs for prevalences were calculated using the *exactci*-function from the PropCIs-package. All analyses were performed using R [27].

Results

Small mammal trapping

During 2017, 1758 small mammals were trapped, including 90 striped field mice, 351 yellow-necked mice (*A. flavicollis*), 61 wood mice (*A. sylvaticus*), 11 field voles, 718 common voles, three European pine voles (*M. subterraneus*), 490 bank voles
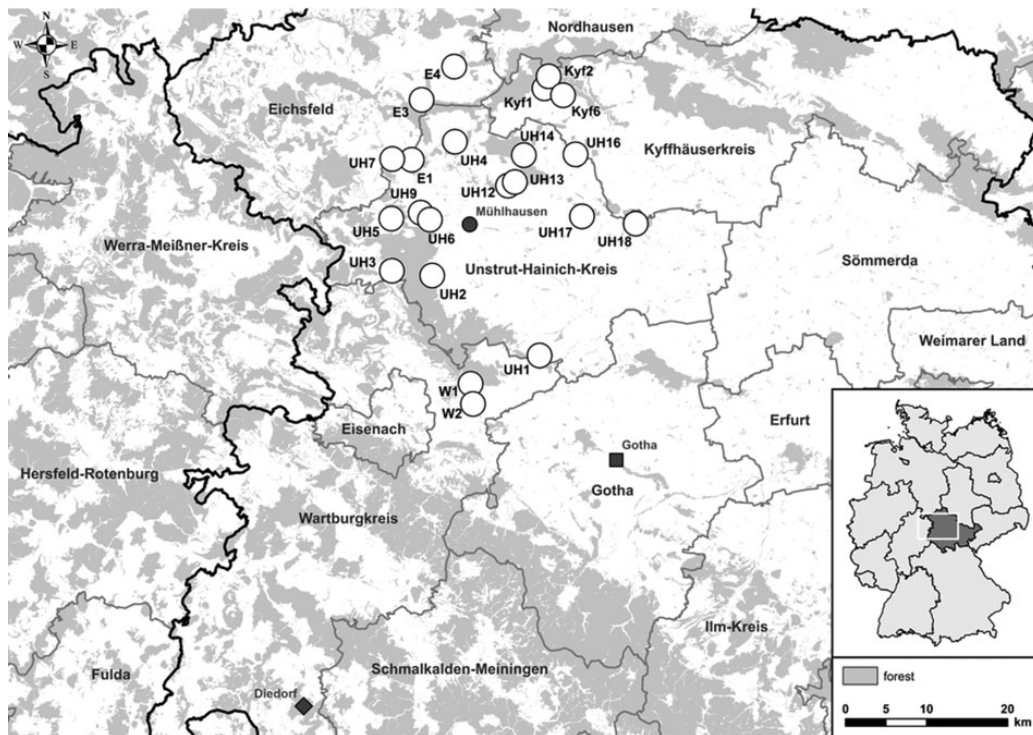
Fig. 1. Map of 22 trap sites around Mühlhausen (black circle) in Thuringia, Germany (see small overview map). Additionally, the sites Diedorf (diamond) and Gotha (square) are shown where previously Puumala orthohantavirus (PUUV) and Tula orthohantavirus (TULV) were detected, respectively.

and 34 shrews including three bicoloured white-toothed shrews (*Crocidura leucodon*), 26 common shrews (*Sorex araneus*), two crowned shrews (*S. coronatus*) and three Eurasian pygmy shrews (*S. minutus*) (Table 1).

Leptospira spp. screening

For 1689 of the 1758 trapped small mammals kidney tissue was available. Overall, 350 of 1689 (20.7%) small mammals tested positive in the *lipL*32 PCR (Table 1). In rodents, the overall prevalence varied between species: field voles (54.5%; 6/11, CI 23.4–83.3%), common voles (30.3%; 205/676, CI 26.9–33.9%), striped field mice (22.1%; 19/86, CI 13.9–32.3%), yellow-necked mice (15.9%; 55/345, CI 12.2–20.2%), wood mice (13.3%; 8/60, CI 5.9–24.6%) and bank voles (11.4%; 54/474, CI 8.7–14.6%). Two of 26 common shrews (7.7%; CI 0.9–25.1%) were tested positive and one of three bicoloured white-toothed shrews was also positive. None of the European pine voles, crowned shrews and Eurasian pygmy shrews tested positive.

The overall prevalence increased from spring (2.2%, 6/278, CI 0.8–4.7%) to summer (22%, 149/678, CI 18.9–25.2%) and fall (26.6%, 195/733, CI 23.4–30.0%). *Leptospira* spp. were detected at 21 of 22 sites with an average site-specific prevalence ranging from 2.4% (2/84, CI 0.3–8.4%) at site UH3 to up to 41.5% (22/53, CI 28.1–55.9%) at site W1. The highest prevalence was measured at site E4 with 76.5% (13/17, CI 50–93.2%) in fall just for common voles. The most abundant genomospecies was *L.*

kirschneri (*n* = 108; 93.1%); *L. borgpetersenii* was found only in a few individuals (*n* = 8, 6.9%); no other genomospecies was identified. Common voles only harboured *L. kirschneri* (*n* = 92; 100%). Similarly, in striped-field mice (*n* = 2), wood mice (*n* = 2), field voles (*n* = 1) and common shrews (*n* = 1) also exclusively *L. kirschneri* was identified. Yellow-necked mice carried *L. kirschneri* (62.5%; 6/9) or *L. borgpetersenii* (37.5%; 3/9), and bank voles also carried *L. kirschneri* (45%; 4/9) or *L. borgpetersenii* (55.5%, 5/9). *L. kirschneri* and *L. borgpetersenii* circulated in the same bank vole population at one site (KYF1) during the same trapping season. Otherwise only a single *Leptospira* genomospecies was detected per site depending on trapping location and species.

Hantavirus screening

TULV-RNA was detected in 13.8% (93/674, CI 11.3–16.6%) of common voles, in none of the 11 field voles and none of the three European pine voles (Table 1). Overall prevalence in common voles was highest in spring with 20.2% (16/79, CI 12.0–30.1%), followed by fall with 16.2% (51/315, CI 12.3–20.7%) and summer with 9.3% (26/280, CI 6.2–13.3%). No TULV-RNA was found at three sites (E3, UH3, UH9; combined 0/24, CI 0.0–14.2%), while prevalences of up to 33.8% (KYF6; 23/68, CI 17.8–37.4%) were detected among sites where at least 10 common voles were tested. The highest prevalence from sites with 10 or more tested common voles was measured in spring at site UH17 with 58.3% (7/12, CI 27.7–84.8%). TULV RNA positive voles originated from 18 of 21 sites where common voles were

Table 1. Small mammals trapped in Thuringia, Germany, and results of *Leptospira* spp. PCR and hantavirus RT-PCR analyses for Dobrava-Belgrade orthohantavirus (DOBV), Tula orthohantavirus (TULV) and Puumala orthohantavirus (PUUV).

| Common name (scientific name)	Total number of animals trapped	*Leptospira* spp. (number of animals tested/total number of animals, prevalence, 95% confidence interval)				Hantavirus (number of animals tested/total number of animals, prevalence, 95% confidence interval)			
		Spring	Summer	Fall	Total	Spring	Summer	Fall	Total
Striped field mouse (*Apodemus agrarius*)	90	0/2	10/53 (18.9%, 9.4–32.0%)	9/31 (29%, 14.2–48.0%)	19/86 (22.1%, 13.9–32.3%)	0/2	0/53	0/31	0/86 No DOBV
Yellow-necked mouse (*Apodemus flavicollis*)	351	0/59	32/154 (20.8%, 14.7–28.0%)	23/132 (17.4%, 11.4–25.0%)	55/345 (15.9%, 12.2–20.2%)	n.d.	n.d.	n.d.	n.d.
Wood mouse (*Apodemus sylvaticus*)	61	1/25 (4%, 0.1–20.4%)	3/15 (20%, 4.3–48.1%)	4/20 (20%, 5.7–43.7%)	8/60 (13.3%, 5.9–24.6%)	n.d.	n.d.	n.d.	n.d.
Field vole (*Microtus agrestis*)	11	0/1	2/5 (40%, 5.3–85.3%)	4/5 (80%, 28.4–99.5%)	6/11 (54.5%, 23.4–83.2%)	0/1	0/5	0/5	0/11
Common vole (*Microtus arvalis*)	718	4/78 (5.1%, 1.4–12.6%)	74/285 (26%, 21.0–31.4%)	127/313 (40.6%, 35.1–46.2%)	205/676 (30.3%, 26.9–33.9%)	16/79 (20.2%, 12.0–30.1%)	26/280 (9.3%, 6.2–13.3%)	51/315 (16.2%, 12.3–20.7%)	93/674 (13.8%, 11.3–16.6%) TULV
European pine vole (*Microtus subterraneus*)	3	0/0	0/2	0/1	0/3	0/0	0/2	0/1	0/3
Bank vole (*Clethrionomys glareolus*)	490	1/112 (0.9%, 0.0–4.9%)	27/158 (17.1%, 11.6–23.9%)	26/204 (12.7%, 8.5–18.1%)	54/474 (11.4%, 8.7–14.6%)	1/111 (1.0%, 0.0–4.9%)	2/157 (1.3%, 0.2–4.5%)	4/203 (2.0%, 0.5–5.0%)	7/471 (1.5%, 0.6–3.0%) PUUV
Bicoloured white-toothed shrew (*Crocidura leucodon*)	3	0/0	0/0	1/3	1/3	n.d.	n.d.	n.d.	n.d.
Common shrew (*Sorex araneus*)	26	0/1	1/6 (16.7%, 0.4–64.1%)	1/19 (5.3%, 0.1–26.0%)	2/26 (7.7%, 0.9–25.1%)	n.d.	n.d.	n.d.	n.d.
Crowned shrew (*Sorex coronatus*)	2	0/0	0/0	0/2	0/2	n.d.	n.d.	n.d.	n.d.
Eurasian pygmy shrew (*Sorex minutus*)	3	0/0	0/0	0/3	0/3	n.d.	n.d.	n.d.	n.d.
Total	1758	6/278 (2.2%, 0.8–4.7%)	149/678 (22%, 18.9–25.2%)	195/733 (26.6%, 23.4–30.0%)	350/1689 (20.7%, 18.8–22.7%)				

n.d., not done.
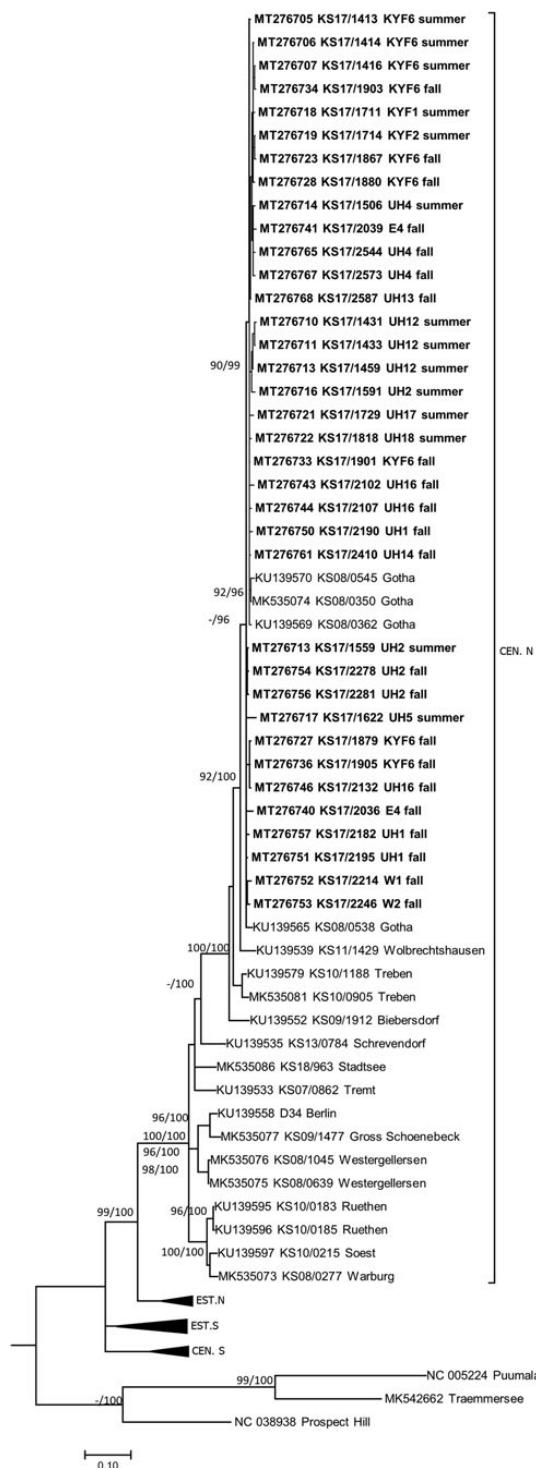
Fig. 2. Consensus phylogenetic tree of the partial S segment sequences of Tula orthohantavirus (TULV) (alignment length 549 nucleotides (nt), positions 406–951, counting according to TULV S segment, accession number NC_005227). TULV is sorted in the clades Central North (CEN.N), Central South (CEN.S), Eastern North (EST.N) and Eastern South (EST.S). The consensus tree is based on Bayesian analyses with 10^7 generations and a burn-in phase of 25%, and maximum-likelihood analyses, with 1000 bootstraps and 50% cut-off using the general time reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter for both algorithms. Posterior probabilities exceeding 95% from Bayesian analyses are given behind and bootstrap values are given before the slash for major nodes if exceeding 75%. The tree reconstructions were done via CIPRES [23]. Alignments were constructed with Bioedit V7.2.3. [21] using the Clustal W Multiple Alignment algorithm implemented in the program. Names in bold indicate newly generated sequences (see Supplementary Table S1). Triangles indicate compressed branches (see Supplementary Table S2 for used sequences). Clade designation followed previous publications for TULV [28].

trapped. TULV was present at only four sites in spring, despite common vole presence at 15 sites. The overall prevalence at these four sites was 50% (16/32, CI 38.9–68.1%). In summer, TULV was present at 14 sites and at 15 sites in fall. The four sites with high prevalences in spring did not differ significantly from the rest in summer ($\chi^2 = 0.031$, $P = 1$) or in autumn ($\chi^2 = 0.474$, $P = 0.57$). Phylogenetic analysis showed that the sequences clustered with TULV sequences from geographically close Gotha, Thuringia, Germany (Fig. 1, square), in the TULV Central North (CEN. N) clade (Fig. 2).

In 1.5% (7/471, CI 0.6–3.0%) of tested bank voles PUUV-RNA was detected. Positive voles were trapped at neighbouring sites UH2, UH3, UH9 and UH6 (Fig. 1). Phylogenetic analysis revealed that the novel PUUV strains belong to the PUUV Central European (CE) clade. The novel sequences clustered closest to sequences from western and northwestern parts of Germany such as Gilserberg (Hesse), Goettingen and Sennickerode (both in Lower Saxony) (Fig. 3). Interestingly, PUUV sequences from Diedorf (Thuringia, Fig. 1, diamond), a site only 50 km away from the trapping locations in this study (Fig. 1), clustered differentially, i.e. with sequences from southern Germany, like Swabian Jura and Bavarian forest.

DOBV infection was not detected in any of the 86 tested striped field mice (Table 1).

Coinfections

In 6.6% (44/671, CI 4.8–8.7%) of common voles, we detected a coinfection of *Leptospira* spp. with TULV. There was no statistical difference between coinfection prevalence detected in forest ecotone (7.7%; 3/39, CI 1.6–20.9%) and in grassland (6.5%; 41/632, CI 4.7–8.7%) ($\chi^2 = 0.0114$, $P = 0.91$). Seasonal differences became apparent. While the prevalence of common voles infected with both pathogens differed significantly ($\chi^2 = 6.563$, $P = 0.01$) between summer 4.3% (CI 2.2–7.4%, 12/280) and fall 10.2% (CI 7.1–14.1%, 32/313), no coinfections were detected in spring (0/78).

The initial global generalised linear mixed model had a R^2_{marginal} of 0.52 and no overdispersion, but the factor *season* was associated with increased multicollinearity (variance inflation factor >4) and was subsequently omitted from the model. Table 2 shows the comparison of candidate models as well as their respective AIC and model weights. The first three models were included in the AIC cut-off value of Δ2 and subject to model averaging. Averaged parameter estimates and respective relative importance are presented in Table 3. Individual coinfection probability with TULV and *Leptospira* spp. was driven by both, individual and population-level factors. Individual age and population-level TULV and *Leptospira* spp. prevalences are sig-
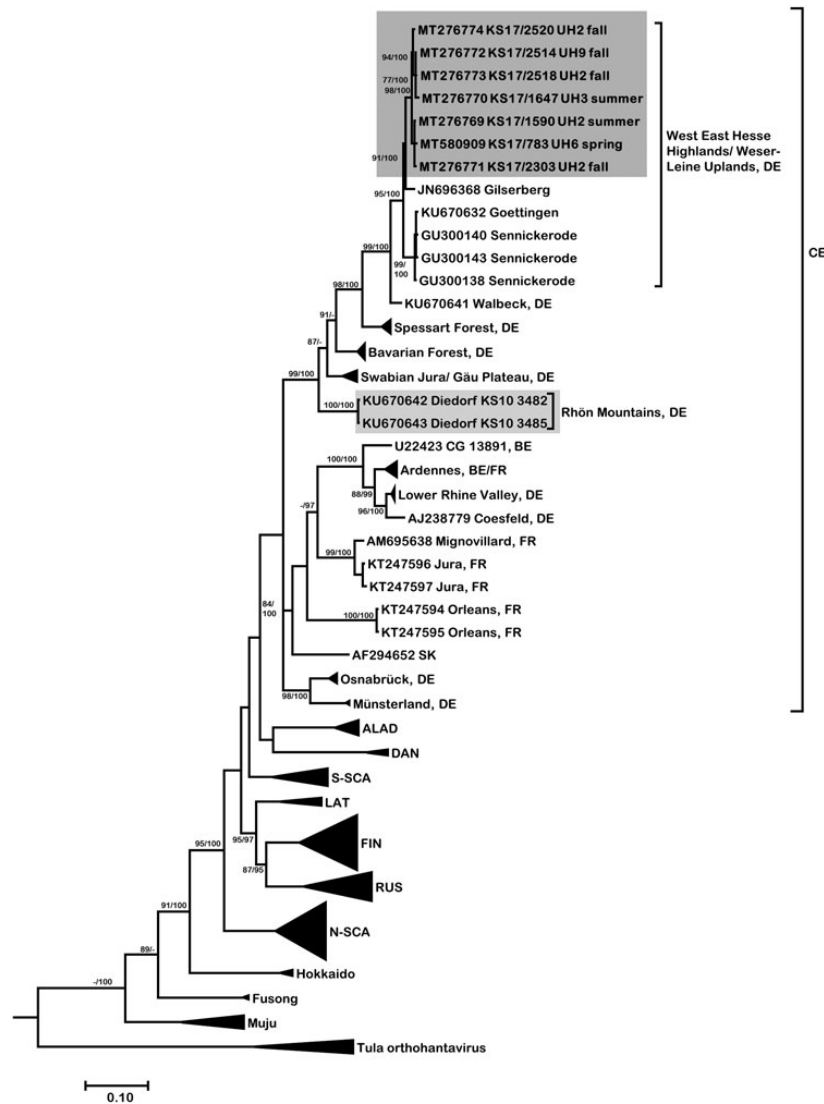
Fig. 3. Consensus phylogenetic tree of partial S segment sequences for Puumala orthohantavirus (PUUV) (alignment length 711 nt, positions 355–1065, counting according to PUUV S segment, accession number NC_005224). PUUV is sorted in the clades Alpe-Adrian (ALAD), Central European (CE) clade including Belgium (BE), France (FR), Germany (DE), Slovakia (SK), Danish (DAN), Finnish (FIN), Latvian (LAT), Northern-Scandinavian (N-SCA), Russian (RUS), Southern-Scandinavian (S-SCA) as well as the PUUV strains Hokkaido, Muju and Fusong. The consensus tree is based on Bayesian analyses with 1.5×10^7 generations and a burn-in phase of 25%, and maximum-likelihood analyses, with 1000 bootstraps and 50% cut-off using the general time reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter for both algorithms. Posterior probabilities exceeding 95% from Bayesian analyses are given behind and bootstrap values are given before the slash for major nodes if exceeding 75%. The tree reconstructions were done via CIPRES [23]. Alignments were constructed with Bioedit V7.2.3. [21] using the Clustal W Multiple Alignment algorithm implemented in the program. Names in bold indicate newly generated sequences (see Supplementary Table S1). Triangles indicate compressed branches (see Supplementary Table S2 for used sequences). Clade designation followed previous publications for PUUV [11, 29].

nificant factors, in determining coinfections. Both abundance measures (delayed and direct) were selected in the averaging process, but only the abundance in the previous season seemed to influence subsequent coinfection dynamics (delayed density dependence). Parameter effect sizes (mean and 95% CI) are shown in Figure 4a. Individual weight had the most dominant effect, while the CIs of the delayed abundance marginally incorporated zero. Model predictions for each factor are shown in Figure 4 (b, c), where for each factor all other factors were kept constant at their respective mean value. Predictions show that older individuals have a higher probability of being coinfected and that a higher abundance of common voles in the previous

Table 2. Binomial generalised linear models explaining the probability of the occurrence of coinfections between *Leptospira* spp. and TULV. Estimates of continuous variables and presence of categorical (indicated by+) population-level and individual variables are presented. Models with Δ AIC >2 were excluded. DF = degrees of freedom, logLik = log-likelihood value

	Epidemiological		Ecological							
		Population			Individual					
Prevalence TULV	Prevalence *Leptospira* spp.	Abundance	Delayed abundance	Sex	Weight	df	logLik	AICc	ΔAICc	Model weight
0.062	0.047		0.033		0.173	6	−93.424	199.1	0	0.343
0.069	0.051				0.151	5	−95.104	200.4	1.3	0.179
0.067	0.046	−0.008	0.033		0.173	7	−93.321	200.9	1.87	0.135
0.062	0.047		0.033	+	0.173	7	−93.392	201.1	2.01	0.126
0.072	0.050	−0.004			0.151	6	−95.068	202.3	3.29	0.066
0.070	0.051			+	0.151	6	−95.085	202.4	3.32	0.065
0.068	0.046	−0.008	0.033	+	0.173	8	−93.291	202.9	3.89	0.049
0.073	0.050	−0.004		+	0.151	7	−95.052	204.4	5.33	0.024
	0.063		0.039		0.161	5	−99.073	208.3	9.24	0.003
	0.066	0.021	0.036		0.163	6	−98.118	208.4	9.39	0.003
0.095			0.049		0.159	5	−99.844	209.8	10.78	0.002
	0.063		0.039	+	0.162	6	−99.071	210.4	11.29	0.001
	0.066	0.021	0.036	+	0.163	7	−98.117	210.5	11.46	0.001
0.102		−0.016	0.046		0.160	6	−99.618	211.4	12.39	0.001
0.095			0.049	+	0.160	6	−99.689	211.6	12.53	0.001
	0.075	0.025			0.139	5	−100.857	211.9	12.81	0.001
	0.070				0.137	4	−102.131	212.4	13.3	0

Table 3. Model averaged estimates for the probability of the occurrence of coinfections between *Leptospira* and TULV. Relative importance as the sum of Akaike weights of all best fitting model where the specific variable is included. Significant factors are highlighted in bold. S.E. = Standard Error

Variable	Estimate	S.E.	*z*-value	*P*-value	Relative importance
Intercept	−10.126	1.425	7.086	**<0.001**	
Prevalence TULV	0.065	0.020	3.264	**0.001**	1.00
Prevalence *Leptospira* spp.	0.048	0.014	3.484	**<0.001**	1.00
Weight	0.167	0.032	5.143	**<0.001**	1.00
Delayed abundance	0.033	0.018	1.864	0.062	0.73
Abundance	−0.008	0.018	0.448	0.654	0.21

season increased the probability of subsequent individual coinfections. For both pathogens an increasing prevalence (while keeping the other pathogen constant) increased the probability of coinfections. As both pathogens differ in their range of detected prevalences, this effect is more prominent in *Leptospira* spp. compared to TULV. However, the relationship between the increase in prevalences of single pathogens and coinfections is significantly better explained by an exponential increase (*Leptospira* spp.: $R^2 = 0.99$; TULV: $R^2 = 0.99$) compared to a linear one (*Leptospira* spp.: $R^2 = 0.86$; TULV: $R^2 = 0.90$) (comparison *Leptospira* spp.: $F = 334.88$; $P < 0.001$, TULV: $F = 451.06$; $P < 0.001$). This indicates that prevalences near the upper end of the potential range result in disproportionally more coinfections

compared to lower prevalences. Two of 469 bank voles (0.4%) tested positive for PUUV and *Leptospira* spp. These originated both from site UH2 in fall.

Discussion

We detected *Leptospira* spp. in several small mammal species in central Germany. Compared to a previous study in the same region (Fig. 1, square) [4], overall prevalence was higher in this study. However, the tendency that *Microtus* spp. had, on average, higher prevalence compared to most other species is mirrored here. In a European context, studies that screened at least 10 individuals of one species, generally reported similar prevalence for
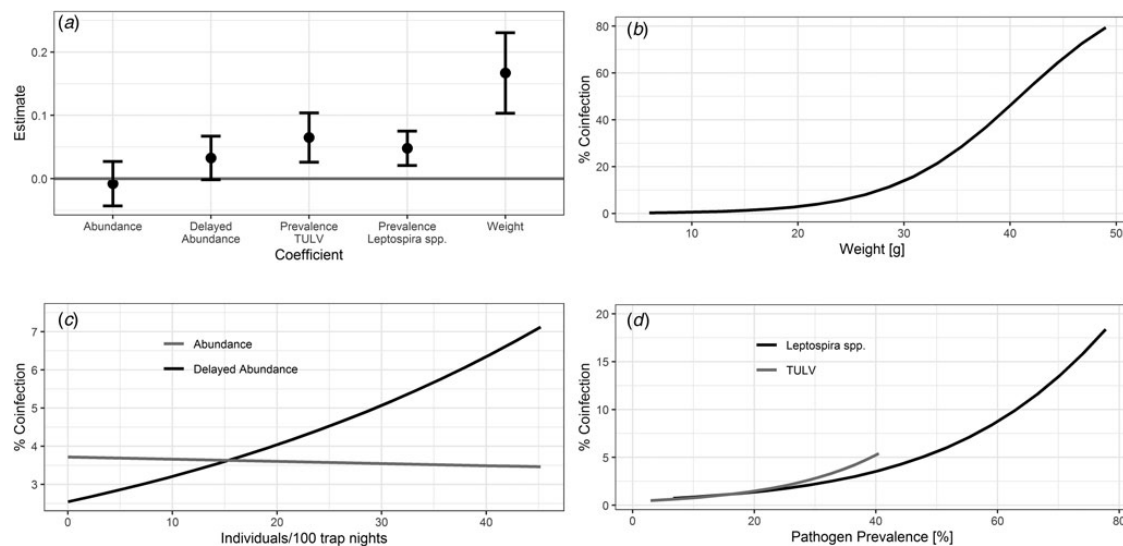
Fig. 4. Graphical representation of the model averaging following multimodel inference. (a) Averaged factor mean estimates and their 95% confidence interval. (b–d) Prediction for each factor in the average model. For each predicted factor all other factors were kept constant at their respective mean value. (b) Relationship between individual weight and prevalence of coinfections. (c) Density dependence (direct and delayed) of coinfections. (d) Relationship between single pathogen infections and the prevalence of coinfections.

striped field mice (12.0–19.6%) [4, 16, 31], common voles (14.0–30.0%) [4, 16, 31–33] and field voles (12.0–30.1%) [4, 31]. For the yellow-necked mouse only a study in Serbia detected a higher average prevalence with 34.3% [33], for the wood mouse studies detected similar average prevalence with 15.4% and 18.0% [31, 34] and for bank voles our study is in line with a previously published prevalence [4, 31].

In general, prevalence increased from spring to fall, likely reflecting more favourable conditions for survival outside the host at high temperature and in moist soil [3]. Interestingly, the strong variance of *Leptospira* spp. prevalence was not only dependent on season but also on site. In fall, the season with the highest overall prevalence, there was high spatial variability in *Leptospira* spp. prevalence. While there was no *Leptospira* spp. at some sites, three sites exhibited >65% prevalence for the common vole in fall (E4, KYF6, W2). A comparable *Leptospira* spp. prevalence is often reported only in Norway rats (*Rattus norvegicus*) collected in sewage systems [35].

High prevalence of *Leptospira* spp. in certain sites arise from local environmental conditions such as soil composition (e.g. mineral and salt composition), soil humidity [36] and the presence of water bodies. Irrigation can be a significant factor for *Leptospira* prevalence in rodents [37] and human outdoor activity, mainly watersports, is related to localised outbreaks of leptospirosis in humans [38, 39]. The effect of livestock on human or even rodent infection risk is still unclear [40] and requires further investigation. On a larger scale, weather effects like intense rainfall with subsequent flooding have been shown to cause more widespread outbreaks of leptospirosis [39]. Further studies should incorporate these risk factors to estimate the spatial persistence of *Leptospira* in their natural reservoirs.

In grassland, prevalence was especially high in common and field voles, which were exclusively infected with *L. kirschneri* [4, 31, 41]. Forest rodents were found to carry either *L. kirschneri*

or *L. borgpetersenii*; *L. interrogans* was not detected here. We detected *L. kirschneri* in wood mice and either *L. kirschneri* or *L. borgpetersenii* in bank voles and yellow-necked mice. Other studies reported lower prevalence for *L. borgpetersenii* but high prevalence for *L. interrogans* in forest rodents [4, 31, 41]. All these studies are consistent with our finding that *L. kirschneri* is the most frequently found *Leptospira* genomospecies in small mammals in Germany [4, 31, 41].

The detection of TULV-RNA at 18 of 21 sites in this study where common voles were trapped is in line with the German-wide distribution of this pathogen [13]. The overall prevalence of 13.9% in common voles is comparable to previously published values of 6.2–23.4% in Europe including Austria, Czech Republic, France, Germany and Hungary [13, 16, 28, 32]. Field voles and European pine voles were not infected with TULV, even though TULV-positive common voles were present in the sites. This finding confirms the common vole to be the main reservoir for TULV and other *Microtus* spp. to be rather accidental hosts [13] even though it is based on a small number of individuals from these two species that were available for analyses. As expected, the sequences clustered in the CEN.N clade of TULV together with sequences from geographically close origin (see [28]).

The very low prevalence of PUUV in this study was most likely a result of the study location at the distributional edge of this hantavirus in Germany. High PUUV prevalence was detected earlier in bank voles during the hantavirus outbreak year 2010 in the western part of Thuringia. Those published PUUV sequences (site Diedorf, see Fig. 1) formed a separate clade 'Rhön Mountains' [11, 30]. Thuringia is situated at the eastern distribution border of PUUV in Germany [11] and features zones with previously reported disease clusters in humans and infected bank voles only in the western part of the state [9, 11, 30], while the exact extent of the distributional range is largely unknown. The presented phylogeny provides further information on the dynamics of PUUV in bank voles along

its distribution border, as sequences from this study did not cluster with sequences from the abovementioned site Diedorf in Thuringia, but instead with sequences from Lower Saxony and Hesse. This observation may suggest two immigration routes of PUUV-infected bank voles into Thuringia over time, which presents an interesting opportunity to study the short- and long-term dynamics of zoonotic pathogens along the edges of their distributional range in the future.

In this study, we did not detect DOBV infections in 86 striped field mice. DOBV infections have been detected only in striped field mice from more eastern and northern located sites, including the eastern part of Thuringia [12, 42]. Likewise, human infections were detected exclusively in eastern and north-eastern Germany [9, 43].

Coinfection with both, *Leptospira* and TULV in common voles were observed before in Hungary with a prevalence of 3.7% [16]. We identified both, individual and population-level factors associated with coinfection of *Leptospira* and hantavirus in common voles. Individual-level drivers seemed to be mostly associated with age. For each pathogen this has been previously described [4, 35, 44]. The possibility of infection increases over each individual's lifetime and common voles are probably persistently infected with both pathogens, although we have to acknowledge that weight might be an imperfect proxy for age, especially when chronically infected, coinfected individuals could potentially suffer from malnourishment.

Overall, coinfections of *Leptospira* spp. and TULV did depend on host density. Rather than coinfections increasing with immediate density, there was a time-lagged response, where individual coinfections were positively correlated to the density 3 months ago. For other pathogens, this time delay has been shown to be an integral part of the transmission process where an increase in density enhances the availability of susceptible hosts that later can become infected [45]. In coinfections, this aspect might even be amplified, as the transmission process for two pathogens has to be completed. The route of transmission can potentially add to the delayed effect. Rodriguez-Pastor *et al.* [46] detected delayed density dependence in *Bartonella rochalimae* and attributed it to the flea life-cycle as a potential cause for the delayed response. In our context, *Leptospira* spp. can survive outside of their host up to 9 weeks in soil [47] and up to 20 months in freshwater [48, 49]. Long periods of environmental survival might preclude any association with immediate host abundance and rather favour delayed responses.

Unsurprisingly, both pathogens are positively associated with increased coinfections, representing the underlying mathematical probability of coinfections to occur when prevalences of both pathogens increase. However, this relationship is best characterised by an exponential regression rather than a linear one (Fig. 4d), indicating that high prevalences are associated with disproportionally more coinfections. This could be interpreted as increased availability of individuals susceptible to coinfections in high prevalence scenarios for both pathogens. Telfer *et al.* [50] highlighted the importance of pathogen community interaction in determining the overall individual susceptibility to subsequent infections. This would imply that an infection with one of the two pathogens would compromise immunocompetency of the infected individual facilitating a 'more efficient' infection with the other pathogen. Our methodology is, however, not suitable to track individual changes within a population across time and might therefore miss subtle individual effects.

Consequently, frequent coinfections were observed in areas where a particularly high prevalence of *Leptospira* spp. was detected. We conclude that, at least for TULV in grassland, high levels of coinfections with *Leptospira* spp. are rather driven by the spatial assemblage of high *Leptospira* spp. prevalences than by TULV prevalence. Despite the low zoonotic potential of TULV [51], coinfections are of general concern. At sites with a high prevalence of *Leptospira* spp. in rodents and an associated increase in human leptospirosis cases, our results suggest that there is also an increased risk of hantavirus coinfections, that might go undetected in humans when coinfections exhibit similar clinical presentations. The spatial assemblage of high *Leptospira* spp. prevalence is therefore of concern as it might also present hot-spots for coinfections with other pathogens. The environmental and epidemiological drivers associated with the patchy occurrence of those hot-spots should be the topic of future research.

References

1. **Thomas V *et al.*** (2001) Coinfection with *Borrelia burgdorferi* and the agent of human granulocytic ehrlichiosis alters murine immune responses, pathogen burden, and severity of Lyme arthritis. *Infection and Immunity* **69**, 3359–3371.

2. **Sunil-Chandra NP *et al.*** (2015) Concomitant leptospirosis-hantavirus co-infection in acute patients hospitalized in Sri Lanka: implications for a potentially worldwide underestimated problem. *Epidemiology and Infection* **143**, 2081–2093.

3. **Levett PN** (2001) Leptospirosis. *Clinical Microbiology Reviews* **14**, 296–326.

4. **Fischer S *et al.*** (2018) *Leptospira* genomospecies and sequence type prevalence in small mammal populations in Germany. *Vector Borne and Zoonotic Diseases* **18**, 188–199.

5. **Haake DA and Levett PN** (2015) Leptospirosis in humans. *Current Topics in Microbiology and Immunology* **387**, 65–97.

6. **Pappas G *et al.*** (2008) The globalization of leptospirosis: worldwide incidence trends. *International Journal of Infectious Diseases* **12**, 351–357.

7. **ICTV** (2020) International Committee on Taxonomy of Viruses (https://ictv.global/taxonomy/).

8. **Jonsson CB, Figueiredo LT and Vapalahti O** (2010) A global perspective on hantavirus ecology, epidemiology, and disease. *Clinical Microbiology Reviews* **23**, 412–441.

9. **Faber M *et al.*** (2019) Molecular and epidemiological characteristics of human Puumala and Dobrava-Belgrade hantavirus infections, Germany, 2001 to 2017. *Euro surveillance* **24**, 1800675.

10. **Robert Koch-Institute**, survstat. Available at https://survstat.rki.de/. Accessed 08 December 2020.

11. **Drewes S *et al.*** (2017) Host-associated absence of human Puumala virus infections in northern and eastern Germany. *Emerging Infectious Diseases* **23**, 83–86.

12. **Schlegel M *et al.*** (2009) Dobrava-Belgrade virus spillover infections, Germany. *Emerging Infectious Diseases* **15**, 2017–2020.

13. **Schmidt S *et al.*** (2016) High genetic structuring of Tula hantavirus. *Archives of Virology* **161**, 1135–1149.

14. **Hofmann J *et al.*** (2021) Tula Virus as Causative Agent of Hantavirus Disease in Immunocompetent Person, Germany. *Emerging Infectious Diseases* **27**, 1234–1237.

15. **Clement J *et al.*** (2014) Leptospirosis versus hantavirus infections in the Netherlands and in Belgium, 2000 to 2014. *Euro surveillance* **19**, 20912.

16. **Kurucz K *et al.*** (2018) Parallel survey of two widespread renal syndrome-causing zoonoses: *Leptospira* spp. and Hantavirus in urban environment, Hungary. *Vector Borne and Zoonotic Diseases* **18**, 200–205.

17. **Görner M** (2009) Atlas der Säugetiere Thüringens, Biologie-Lebensräume-Verbreitung-Gefährdung-Schutz. Jena.

18. **Mayer-Scholl A *et al.*** (2011) Comparison of two PCR systems for the rapid detection of *Leptospira* spp. from kidney tissue. *Current Microbiology* **62**, 1104–1106.

19. **Essbauer S *et al.*** (2006) A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. *Epidemiology and Infection* **134**, 1333–1344.

20. **Klempa B** (2004) Dobrava and Tula hantaviruses from Central Europe: molecular evolution and pathogenic relevance [dissertation] (Humboldt-Universität zu Berlin).

21. **Hall TA** (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic Acids Symposium Series, pp. 95–98.

22. **Posada D** (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256.

23. **Miller MA, Pfeiffer W and Schwartz T** (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: 2010 gateway computing environments workshop (GCE): IEEE, pp. 1–8.

24. **Price MN, Dehal PS and Arkin AP** (2010) FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**, e9490.

25. **Ronquist F *et al.*** (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

26. **Morris P** (1972) A review of mammalian age determination methods. *Mammal Review* **2**, 69–104.

27. **R Core Team** (2019) *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

28. **Saxenhofer M *et al.*** (2019) Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus.. *Plos Biology* **17**, e3000142.

29. **Castel G *et al.*** (2015) Complete Genome and Phylogeny of Puumala Hantavirus Isolates Circulating in France. *Viruses* **7**(10), 5476–5488.

30. **Faber M *et al.*** (2013) Puumala virus outbreak in Western Thuringia, Germany, 2010: epidemiology and strain identification. *Zoonoses and Public Health* **60**, 549–554.

31. **Mayer-Scholl A *et al.*** (2014) *Leptospira* spp. in rodents and shrews in Germany. *International Journal of Environmental Research and Public Health* **11**, 7562–7574.

32. **Schmidt S *et al.*** (2014) Multiple infections of rodents with zoonotic pathogens in Austria. *Vector Borne and Zoonotic Diseases* **14**, 467–475.

33. **Blagojevic J *et al.*** (2019) The prevalence of pathogenic forms of *Leptospira* in natural populations of small wild mammals in Serbia. *Acta Veterinaria Hungarica* **67**, 338–346.

34. **Vitale M *et al.*** (2018) Human leptospirosis cases in Palermo Italy. The role of rodents and climate. *Journal of Infection and Public Health* **11**, 209–214.

35. **Krojgaard LH *et al.*** (2009) High prevalence of *Leptospira* spp. in sewer rats (*Rattus norvegicus*). *Epidemiology and Infection* **137**(11), 1586–1592.

36. **Thibeaux R *et al.*** (2018) Biodiversity of environmental *Leptospira*: improving identification and revisiting the diagnosis. *Frontiers in Microbiology* **9**, 816.

37. **Morand S *et al.*** (2019) Changing landscapes of Southeast Asia and rodent-borne diseases: decreased diversity but increased transmission risks. *Ecological Applications* **29**, e01886.

38. **Jansen A *et al.*** (2005) Leptospirosis in Germany, 1962–2003. *Emerging Infectious Diseases* **11**, 1048–1054.

39. **Lau CL *et al.*** (2010) Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**, 631–638.

40. **Mwachui MA *et al.*** (2015) Environmental and behavioural determinants of leptospirosis transmission: a systematic review. *PLoS Neglected Tropical Diseases* **9**, e0003843.

41. **Obiegala A *et al.*** (2016) Prevalence and genotype allocation of pathogenic *Leptospira* species in small mammals from various habitat types in Germany. *PLoS Neglected Tropical Diseases* **10**, e0004501.

42. **Rasche FM *et al.*** (2015) Autochthonous Dobrava-Belgrade virus infection in Eastern Germany. *Clinical Nephrology* **83**, 111–116.

43. **Hofmann J *et al.*** (2014) Hantavirus disease in Germany due to infection with Dobrava-Belgrade virus genotype Kurkino. *Clinical Microbiology and Infection* **20**, 648–655.

44. **Khalil H *et al.*** (2016) Declining ecosystem health and the dilution effect. *Scientific Reports* **6**, 31314.

45. **Burthe S *et al.*** (2008) Cowpox virus infection in natural field vole *Microtus agrestis* populations: significant negative impacts on survival. *Journal of Animal Ecology* **77**, 110–119.

46. **Rodríguez-Pastor R *et al.*** (2019) Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. *Parasitology* **146**, 389–398.

47. **Thibeaux R *et al.*** (2017) Seeking the environmental source of leptospirosis reveals durable bacterial viability in river soils. *PLoS Neglected Tropical Diseases* **11**, e0005414.

48. **Andre-Fontaine G, Aviat F and Thorin C** (2015) Waterborne leptospirosis: survival and preservation of the virulence of pathogenic *Leptospira* spp. in fresh water. *Current Microbiology* **71**, 136–142.

49. **Casanovas-Massana A *et al.*** (2018) Quantification of *Leptospira interrogans* survival in soil and water microcosms. *Applied and Environmental Microbiology* **84**, e00507-18.

50. **Telfer S *et al.*** (2010) Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population. *Science* **330**(6001), 243–246.

51. **Reynes JM *et al.*** (2015) Tula hantavirus infection in a hospitalised patient, France, June 2015. *Euro surveillance* **20**, 30095.

Supplementary Table S1 List of all hantavirus sequences generated during this study.

Sample ID	Species	Date captured	Area	Hantavirus	Accession number	Sequence identical to
KS17/1137	*M. arvalis*	10.08.2017	E1	TULV	MT276704	
KS17/2036	*M. arvalis*	23.10.2017	E4	TULV	MT276740	MT276742
KS17/2039	*M. arvalis*	24.10.2017	E4	TULV	MT276741	
KS17/2043	*M. arvalis*	25.10.2017	E4	TULV	MT276742	MT276740
KS17/1711	*M. arvalis*	17.08.2017	KYF1	TULV	MT276718	MT276739
KS17/1963	*M. arvalis*	24.10.2017	KYF1	TULV	MT276739	MT276718
KS17/1714	*M. arvalis*	17.08.2017	KYF2	TULV	MT276719	MT276720
KS17/1716	*M. arvalis*	17.08.2017	KYF2	TULV	MT276720	MT276719
KS17/1413	*M. arvalis*	16.08.2017	KYF6	TULV	MT276705	MT276708
KS17/1414	*M. arvalis*	16.08.2017	KYF6	TULV	MT276706	MT276724, MT276731, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1416	*M. arvalis*	16.08.2017	KYF6	TULV	MT276707	MT276709, MT276763, MT276763
KS17/1417	*M. arvalis*	16.08.2017	KYF6	TULV	MT276708	MT276705
KS17/1420	*M. arvalis*	16.08.2017	KYF6	TULV	MT276709	MT276707, MT276763, MT276763
KS17/1867	*M. arvalis*	26.10.2017	KYF6	TULV	MT276723	MT276726
KS17/1874	*M. arvalis*	24.10.2017	KYF6	TULV	MT276725	MT276706, MT276724, MT276731, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1878	*M. arvalis*	24.10.2017	KYF6	TULV	MT276726	MT276723
KS17/1879	*M. arvalis*	24.10.2017	KYF6	TULV	MT276727	
KS17/1880	*M. arvalis*	24.10.2017	KYF6	TULV	MT276728	
KS17/1881	*M. arvalis*	24.10.2017	KYF6	TULV	MT276729	MT276706, MT276724, MT276731, MT276725, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1882	*M. arvalis*	24.10.2017	KYF6	TULV	MT276730	MT276706, MT276724, MT276731, MT276725, MT276729, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1886	*M. arvalis*	25.10.2017	KYF6	TULV	MT276724	MT276706, MT276731, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1888	*M. arvalis*	25.10.2017	KYF6	TULV	MT276731	MT276706, MT276724, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1889	*M. arvalis*	25.10.2017	KYF6	TULV	MT276732	MT276706, MT276724, MT276731, MT276725, MT276729, MT276730, MT276731, MT276737, MT276738, MT276762
KS17/1901	*M. arvalis*	26.10.2017	KYF6	TULV	MT276733	
KS17/1903	*M. arvalis*	26.10.2017	KYF6	TULV	MT276734	MT276735

Supplementary Table S1 (continued)

Sample ID	Species	Date captured	Area	Hantavirus	Accession number	Sequence identical to
KS17/1904	*M. arvalis*	26.10.2017	KYF6	TULV	MT276735	MT276734
KS17/1905	*M. arvalis*	26.10.2017	KYF6	TULV	MT276736	
KS17/1911	*M. arvalis*	26.10.2017	KYF6	TULV	MT276737	MT276706, MT276724, MT276731, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/1912	*M. arvalis*	26.10.2017	KYF6	TULV	MT276738	MT276706, MT276724, MT276731, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738, MT276762
KS17/2182	*M. arvalis*	27.10.2017	UH1	TULV	MT276747	MT276748, MT276749
KS17/2184	*M. arvalis*	28.10.2017	UH1	TULV	MT276748	MT276747, MT276749
KS17/2185	*M. arvalis*	28.10.2017	UH1	TULV	MT276749	MT276747, MT276748
KS17/2190	*M. arvalis*	29.10.2017	UH1	TULV	MT276750	
KS17/2195	*M. arvalis*	29.10.2017	UH1	TULV	MT276751	
KS17/744	*M. arvalis*	13.05.2017	UH2	TULV	MT276692	
KS17/1559	*M. arvalis*	13.08.2017	UH2	TULV	MT276715	MT276760
KS17/1591	*M. arvalis*	11.08.2017	UH2	TULV	MT276716	
KS17/2278	*M. arvalis*	28.10.2017	UH2	TULV	MT276754	MT276755, MT276757, MT276759
KS17/2280	*M. arvalis*	28.10.2017	UH2	TULV	MT276755	MT276754, MT276757, MT276759
KS17/2281	*M. arvalis*	28.10.2017	UH2	TULV	MT276756	MT276758
KS17/2282	*M. arvalis*	28.10.2017	UH2	TULV	MT276757	MT276754, MT276755, MT276759
KS17/2284	*M. arvalis*	29.10.2017	UH2	TULV	MT276758	MT276756
KS17/2287	*M. arvalis*	29.10.2017	UH2	TULV	MT276759	MT276754, MT276755, MT276757
KS17/2292	*M. arvalis*	29.10.2017	UH2	TULV	MT276760	KS17/1559
KS17/1506	*M. arvalis*	12.08.2017	UH4	TULV	MT276714	
KS17/2532	*M. arvalis*	18.10.2017	UH4	TULV	MT276763	MT276707, MT276709, MT276764
KS17/2539	*M. arvalis*	19.10.2017	UH4	TULV	MT276764	MT276707, MT276709, MT276763
KS17/2544	*M. arvalis*	20.10.2017	UH4	TULV	MT276765	MT276766
KS17/2545	*M. arvalis*	20.10.2017	UH4	TULV	MT276766	MT276765
KS17/2573	*M. arvalis*	20.10.2017	UH4	TULV	MT276767	
KS17/788	*M. arvalis*	09.05.2017	UH5	TULV	MT276693	MT276694, MT276695
KS17/789	*M. arvalis*	09.05.2017	UH5	TULV	MT276694	MT276693, MT276695
KS17/792	*M. arvalis*	10.05.2017	UH5	TULV	MT276695	MT276693, MT276694
KS17/793	*M. arvalis*	10.05.2017	UH5	TULV	MT276696	MT276697, MT276717
KS17/794	*M. arvalis*	10.05.2017	UH5	TULV	MT276697	MT276696, MT276717
KS17/1622	*M. arvalis*	11.08.2017	UH5	TULV	MT276717	MT276696, MT276697
KS17/781	*M. arvalis*	10.05.2017	UH7	TULV	MT276691	
KS17/1431	*M. arvalis*	15.08.2017	UH12	TULV	MT276710	
KS17/1433	*M. arvalis*	15.08.2017	UH12	TULV	MT276711	MT276712
KS17/1457	*M. arvalis*	13.08.2017	UH12	TULV	MT276712	MT276711
KS17/1459	*M. arvalis*	13.08.2017	UH12	TULV	MT276713	
KS17/2587	*M. arvalis*	22.10.2017	UH13	TULV	MT276768	
KS17/2410	*M. arvalis*	18.10.2017	UH14	TULV	MT276761	

Supplementary Table S1 (continued)

Sample ID	Species	Date captured	Area	Hantavirus	Accession number	Sequence identical to
KS17/2418	*M. arvalis*	20.10.2017	UH14	TULV	MT276762	MT276706, MT276724, MT276731, MT276725, MT276729, MT276730, MT276731, MT276732, MT276737, MT276738
KS17/2102	*M. arvalis*	26.10.2017	UH16	TULV	MT276743	
KS17/2107	*M. arvalis*	26.10.2017	UH16	TULV	MT276744	MT276745
KS17/2131	*M. arvalis*	28.10.2017	UH16	TULV	MT276745	MT276744
KS17/2132	*M. arvalis*	26.10.2017	UH16	TULV	MT276746	
KS17/875	*M. arvalis*	11.05.2017	UH17	TULV	MT276698	MT276701, MT276702
KS17/878	*M. arvalis*	12.05.2017	UH17	TULV	MT276699	MT276700, MT276703, MT276721
KS17/879	*M. arvalis*	12.05.2017	UH17	TULV	MT276700	MT276699, MT276703, MT276721
KS17/880	*M. arvalis*	12.05.2017	UH17	TULV	MT276701	MT276698, MT276702
KS17/881	*M. arvalis*	12.05.2017	UH17	TULV	MT276702	MT276698, MT276701
KS17/882	*M. arvalis*	12.05.2017	UH17	TULV	MT276703	MT276699, MT276700, MT276721
KS17/1729	*M. arvalis*	17.08.2017	UH17	TULV	MT276721	MT276699, MT276700, MT276703
KS17/1818	*M. arvalis*	17.08.2017	UH18	TULV	MT276722	
KS17/2214	*M. arvalis*	29.10.2017	W1	TULV	MT276752	
KS17/2246	*M. arvalis*	28.10.2017	W2	TULV	MT276753	
KS17/783	*C. glareolus*	09.05.2017	UH6	PUUV	MT580909	
KS17/1590	*C. glareolus*	11.08.2017	UH2	PUUV	MT276769	
KS17/2303	*C. glareolus*	29.10.2017	UH2	PUUV	MT276771	
KS17/1647	*C. glareolus*	11.08.2017	UH3	PUUV	MT276770	
KS17/2514	*C. glareolus*	24.10.2017	UH9	PUUV	MT276772	
KS17/2518	*C. glareolus*	27.10.2017	UH2	PUUV	MT276773	
KS17/2520	*C. glareolus*	28.10.2017	UH2	PUUV	MT276774	

Supplementary Table S2 List of all sequences used in condensed clades in Fig. 2 and Fig. 3. Lineage nomenclature after [1,2].

Hantavirus	Clade/strain	Accession number
Tula orthohantavirus	Central South (CEN.S)	MK386133 MK386134 MK386135 MK386138 MK386139
	Eastern North (EST.N)	AF063892 KU139555 KU139563 MK535078 MK535083
	Eastern South (EST.S)	MK386130 MK386131 MK386132 MK386136 MK386137 MK386140 Z69991
Puumala orthohantavirus	Alpe-Adrian (ALAD)	AJ314600 AJ314601 AJ888751 AJ888752 FN37782a FN377822 KC676609 KC676613
	CE (Central European)	Ardennes: AJ277031 - AJ277076, AJ277030, KT247593 Bavarian Forest: AY954722, DQ016432, EU439968, EU439969, EU439972, JN696374 - JN696376 Lower Rhine Valley: DQ408268, DQ408271 - DQ408275, KU670633 Münsterland: JN696362, L36943 Osnabrück: JN696355, JN696358, KU670635, KU670640, KJ994776 Spessart Forest: EU246962, JN696356, JN696359, JN696367, JN696371 - JN696373 Swabian Jura/ Gäu Plateaus DQ094844, EU085558, EU085563, EU085565, JN696361, KU670631

Supplementary Table S2 (continued)

Hantavirus	Clade/strain	Accession number
Puumala ortohantavirus	Danish (DAN)	AJ238791
		AJ238792
		AJ238793
	Finnish (FIN)	AF367064
		AF367065
		AF367066
		AF367068
		AF367069
		AF367070
		AF367071
		AJ238788
		AJ238789
		AJ238790
		AJ314597
		HE801633
		JQ319171
		JQ319163
		JQ319166
		JQ319167
		JQ319168
		JQ319161
		JQ319164
		JQ319170
		JQ319162
		JN831950
		Z30702
		Z30704
		Z46942
		Z69985
	Fusong	EF442087
		EF442091
	Hokkaido	AB010730
		AB010731
		AB675465
	Latvian (LAT)	JN657228
		KX757839
		KX757840
	Muju	DQ138128
		DQ138133
		DQ138140
		DQ138142
		JX028273
		JX046484
		JX046487

Supplementary Table S2 (continued)

Hantavirus	Clade/strain	Accession number
Puumala orthohantavirus	Northern-Scandinavian (N-SCA)	AJ223371
		AM746297
		AM746298
		AM746311
		AM746315
		AM746316
		AM746317
		AM746318
		AM746319
		AM746320
		AM746321
		AM746325
		AM746329
		AM746330
		AM746331
		AM746332
		AM746333
		AY526219
		GQ339473
		GQ339474
		GQ339476
		GQ339477
		GQ339478
		GQ339479
		GQ339480
		GQ339481
		GQ339482
		J223380
		U14137
		Z48586
	Russian (RUS)	AB433845
		AJ314598
		AJ314599
		AF442613
		JN657230
		JN657231
		L08804
		M32750
		Z21497
		Z30706
		Z30707
		Z30708
		Z84204

Supplementary Table S2 (continued)

Hantavirus	Clade/strain	Accession number
Puumala orthohantavirus	Southern-Scandinavian (S-SCA)	GQ339483 GQ339484 GQ339485 GQ339486 GQ339487 AJ223368 AJ223369 AJ223376
Tula orthohantavirus	Outgroup sequences	AF017659 AF063892 AF164093 AJ223600 AM945877 Y13979 Z69991

Literature

1. Saxenhofer M, Schmidt S, Ulrich RG, Heckel G (2019) Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus. PLoS Biology 17 (2):e3000142. doi:10.1371/journal.pbio.3000142
2. Castel G, Couteaudier M, Sauvage F, Pons JB, Murri S, Plyusnina A, Pontier D, Cosson JF, Plyusnin A, Marianneau P, Tordo N (2015) Complete Genome and Phylogeny of Puumala Hantavirus Isolates Circulating in France. Viruses 7 (10):5476-5488. doi:10.3390/v7102884
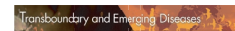
3.5   Paper V

Jeske K, Herzig-Straschil B, Raileanu C, Kunec D, Tauchmann O, Emirhar D, Schmidt S, Trimpert J, Silaghi C, Heckel G, Ulrich RG, Drewes S. Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria. Transboundary and Emerging Diseases (in revision)

Check for updates

SHORT COMMUNICATION

Transboundary and Emerging Diseases   **WILEY**

Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria

Kathrin Jeske[1,2] (ID) | Barbara Herzig-Straschil[3] | Cristian Răileanu[4] | Dusan Kunec[5] (ID) | Oliver Tauchmann[4] | Duygu Emirhar[6] | Sabrina Schmidt[1,7] | Jakob Trimpert[5] (ID) | Cornelia Silaghi[4] | Gerald Heckel[8,9] (ID) | Rainer G. Ulrich[1] (ID) | Stephan Drewes[1] (ID)

[1]Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

[2]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

[3]Naturhistorisches Museum Wien, Vienna, Austria

[4]Institute of Infectology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

[5]Department of Veterinary Medicine, Institute of Virology, Freie Universität Berlin, Berlin, Germany

[6]Department for Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany

[7]German Society of Tissue Transplantation, Essen, Germany

[8]Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

[9]Swiss Institute of Bioinformatics, Lausanne, Switzerland

Correspondence
Rainer G. Ulrich, Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald - Insel Riems, Germany.
Email: rainer.ulrich@fli.de

Abstract

The striped field mouse (*Apodemus agrarius*) is known to carry several zoonotic pathogens, including *Leptospira* spp. and Dobrava–Belgrade orthohantavirus (DOBV). Since its first detection in 1996 in south-east Austria, the striped field mouse has further expanded its range in Austria. Here, we screened 35 striped field mice collected in an Austrian region near the Hungarian border for DOBV, *Leptospira* spp. and seven vector-borne pathogens. Hantavirus RT-PCR screening and DOBV IgG ELISA analysis led to the detection of two DOBV-positive striped field mice. The complete coding sequences of all three genome segments of both strains were determined by a combination of target enrichment and next-generation sequencing. Both complete coding S segment sequences clustered within the DOBV genotype Kurkino clade with the highest similarity to a sequence from Hungary. In one of 35 striped field mice, *Leptospira borgpetersenii* sequence type (ST) 146 was detected. *Bartonella* spp., *Borrelia miyamotoi* and *Neoehrlichia mikurensis* DNA was detected in four, one and two of 32 mice, respectively. *Babesia*, *Anaplasma*, *Ehrlichia* and *Rickettsia* specific DNA was not detected. Future investigations will have to determine the prevalence and invasion of these pathogens with the ongoing range expansion of the striped field mouse in Austria.

KEYWORDS

Apodemus agrarius, *Bartonella*, *Borrelia*, Dobrava–Belgrade orthohantavirus, *Leptospira* spp., *Neoehrlichia mikurensis*, *Rickettsia*

1 | INTRODUCTION

Rodent- and vector-borne diseases are emerging zoonoses. Hantavirus disease and leptospirosis are important, worldwide occurring rodent-borne zoonotic diseases which are hard to differentiate clinically from each other (Kaya et al., 2019; Meerburg et al., 2009). Leptospirosis with a case fatality rate of up to 15% is caused by pathogenic *Leptospira* spp. that are transmitted directly through urine of infected rodents or indirectly via contaminated water bodies (Fischer et al., 2018). Hantavirus disease in Europe, that is haemorrhagic fever with renal syndrome (HFRS), can be caused by different orthohantaviruses (Vaheri et al., 2013). Usually, each orthohantavirus is associated with a distinct rodent species and is transmitted via virus contaminated aerosols or bites. In Europe, most disease cases are caused by Puumala orthohantavirus (PUUV), harboured by the bank vole (*Clethrionomys glareolus* syn. *Myodes glareolus*), and Dobrava–Belgrade orthohantavirus (DOBV) (Vaheri et al., 2013). DOBV is divided into four genotypes that are associated with different *Apodemus* species, and cause different disease courses and case fatality rates (Klempa et al., 2013). Genotype Kurkino is associated with the striped field mouse (*Apodemus agrarius*), whereas genotype Dobrava is carried by the yellow-necked mouse (*Apodemus flavicollis*) (Klempa et al., 2013).

Rodents play also an important role in the life cycle of vector-borne pathogens (Tomassone et al., 2017). Lyme borreliosis is caused by members of the *Borrelia burgdorferi* sensu lato group, especially *B. afzelii* in Europe, which is carried by *Ixodes ricinus* ticks and their rodent hosts (Richter et al., 2004). Furthermore, the relapsing fever agent *Borrelia miyamotoi* is also carried by *I. ricinus* (Siński et al., 2016). *Bartonella* spp. are gram-negative facultative intracellular bacteria, infecting mammalian erythrocytes and endothelial cells (Breitschwerdt, 2014). From these, some can cause human disease, while most *Bartonella* spp. have an unknown zoonotic potential (Gutiérrez et al., 2015). *Rickettsia* spp. are obligate intracellular bacteria and several of them can cause disease in humans with different arthropods described as vectors (Blanco & Oteo, 2006). Several members of the *Ehrlichia* spp. and *Anaplasma* spp. can cause febrile disease in humans, called ehrlichiosis and anaplasmosis, respectively (Ismail et al., 2010), whereas *Neoehrlichia mikurensis* can cause inflammatory infection mainly in immunocompromised patients and is asymptomatic in immunocompetent patients (Portillo et al., 2018). Infection with the protozoan *Babesia microti* can lead to babesiosis with possible persistent infection and malaria-like symptoms. A case fatality rate of up to 20% is observed in immunocompromised patients (Bloch et al., 2019).

In Austria, human infections with hantaviruses and *Leptospira interrogans* are notifiable and up to 90 and 69 cases were reported per year, respectively (Bundesministerium für Arbeit Soziales Gesundheit und Konsumentenschutz, 2019). Human PUUV and leptospirosis cases have been reported in different parts of Austria (Aberle, 2019; Hoenigl et al., 2014). Recently, first autochthonous DOBV infections have been documented in Austria (Aberle, 2019). Reservoir investigations confirmed PUUV in bank voles and Tula orthohantavirus (TULV) in common voles (*Microtus arvalis*) (Bowen et al., 1997; Schmidt et al., 2014). *Leptospira* DNA was detected previously in bank vole, common vole, yellow-necked mouse and wood mouse but without genomospecies identification and sequence type (ST) determination (Schmidt et al., 2014). *Borrelia miyamotoi* and *N. mikurensis* were reported in Austrian patients before (Glatz et al., 2014; Tobudic et al., 2020). In addition, *Rickettsia*, *Borrelia* and *Bartonella* DNA was detected in voles and mice from Austria (Schmidt et al., 2014).

The striped field mouse was first reported from Bad Radkersburg in south-eastern Styria in 1996. In 2003, it was detected at Lake Neusiedl, the Hanság area in northern Burgenland at the border to Slovenia and Hungary (Sackl et al., 2007; Spitzenberger, 1997). By 2013, the striped field mouse had colonized an area of about 140 km in length and 56 km in width along the Slovakian, Hungarian and Slovenian border (Spitzenberger & Engelberger, 2014). The ongoing range expansion of the striped field mouse in Austria raised the question whether this species represents a reservoir for zoonotic pathogens in the newly colonized area. Therefore, the objective of this study was a retrospective screening of striped field mice from Austria for the presence of DOBV, *Leptospira* spp. and several vector-borne pathogens and their sequence determination and/or sequence typing.

2 | MATERIALS AND METHODS

Thirty-five striped field mice were trapped at 13 sites in Austria in 2011 and 2017 (Figure 1a). Dissection of mice, RNA and DNA extraction and RT-PCR and PCR followed standard procedures (for methodological details, see Appendix S1). For 35 mice, kidney and chest cavity fluid samples were obtained, whereas for 32 animals, also spleen and liver tissue could be obtained. For 34 mice, lung tissue was available for hantavirus screening; for the remaining mouse, liver tissue was used.

The complete coding sequences (CDS) of all three genome segments of DOBV strains were determined by a combination of target enrichment and high-throughput sequencing. Sequencing libraries were constructed using the NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs). DOBV-derived libraries were captured by in solution hybridization with a custom-made target enrichment myBaits array (Arbor Biosciences). The captured libraries were then analysed by high-throughput sequencing on an Illumina MiSeq platform, and DOBV sequences were determined by mapping the DOBV reads to the closest available reference genome using Geneious Prime suite (https://www.geneious.com). Additionally, a primer-walking approach was used to determine the complete S and M segment CDS of strain KS18/1812 (see Appendix S1).

The serological detection of DOBV-reactive antibodies in chest cavity fluid samples of mice used an established IgG ELISA with a recombinant DOBV nucleocapsid protein as antigen (see Appendix S1).
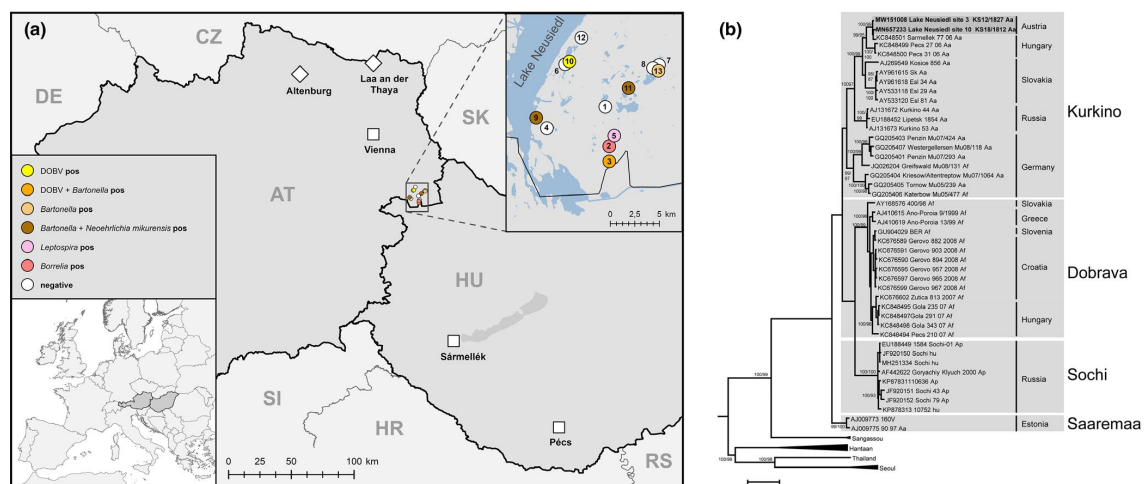
FIGURE 1 Trapping sites in Austria with two inserts showing the location in Europe and the magnified area with numbered sites (a) and consensus phylogenetic tree of Dobrava–Belgrade orthohantavirus (DOBV) based on partial nucleocapsid protein-coding S segment sequences (b). Trapping sites where previously other rodents were collected (Schmidt et al., 2014) and investigated for *Leptospira* genomospecies and sequence types determined here are marked with empty rhombs (a). The 13 trapping sites were classified into three regions: A: sites 4, 6, 9, 10 and 12, B: sites 2, 3 and 5 and C: sites 1, 7, 8, 11 and 13; see Table S1. (b) Classification of DOBV genotypes is given as previously suggested (Klempa et al., 2013). General time reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter was used for tree reconstruction. The consensus tree is based on Bayesian analyses with 10,000,000 generations and a burn-in phase of 25%. Bootstrap values and posterior probabilities are only given if branches are supported with values above 75 and 0.95 and if branches of both trees were consistent. Sangassou (JQ082303, JQ082300), Hantaan (AF288646, EU02220, FJ753396, GU329991, JQ665905, KC844227), Thailand (AM397664) and Seoul orthohantavirus strains (AY006465, GU361893, GU592938, GU592943) were used as outgroup for (b). Aa, *Apodemus agrarius*; Af, *Apodemus flavicollis*; Ap, *Apodemus ponticus*; AT, Austria; CZ, Czech Republic; DE, Germany; HR, Croatia; Hu, *human*; HU, Hungary; RS, Serbia; SI, Slovenia; SK, Slovakia

3 | RESULTS

Screening of lung (or liver) derived RNA with RT-PCR assays targeting the S segment of DOBV resulted in the detection of two positive samples of 35 mice investigated (Table S1). Both partial S segment sequences clustered within the DOBV genotype Kurkino clade with the highest similarity to a sequence from Sarmellek, Hungary (Figure 1b). DOBV IgG ELISA analysis of chest cavity fluid samples of all striped field mice detected only the two RT-PCR-positive animals as seropositive (Table S1).

Target enrichment-based high-throughput sequencing resulted in the determination of the complete CDS of all three genome segments of both DOBV strains. The obtained sequences did not differ from sequences obtained by parallel primer-walking-based approach. The S segment CDS of both samples confirmed the affiliation with genotype Kurkino and the high similarity to the sequences from Hungary, and the sequence from Sarmellek in particular (Figure S1A). Currently, no M and L segment sequences of Hungarian DOBV strains are available in GenBank. However, as expected, the obtained complete CDS of M and L segments showed the highest similarity to sequences of DOBV Kurkino from western Slovakia, which is geographically adjacent to the investigated region (Figure S1B,C).

Screening of kidney tissue for *Leptospira* DNA by conventional *lipl32*-PCR resulted in the detection of one positive animal from trapping site 5 (Figure 1a; Table S1). The more specific *secY*-PCR-based genomospecies determination and multilocus sequence typing (MLST) showed that the affected mouse was infected with *L. borgpetersenii*, sequence type (ST) 146. The analysis of previously detected *Leptospira* DNA-positive small mammals from Lower Austria (Schmidt et al., 2014) resulted in the identification of *L. kirschneri* ST 110 in one common vole and one bank vole, as well as a new ST of *L. interrogans* (ST 297) in a yellow-necked mouse.

PCR screening of 32 striped field mice resulted in the detection of *Bartonella*, *Borrelia*, and *N. mikurensis*-specific DNA. On the other hand, PCR screening did not confirm presence of *Babesia*, *Anaplasma*, *Ehrlichia* or *Rickettsia* DNA in the analysed samples (Table S1). The sequencing of obtained PCR products showed that striped field mice were infected with *Bartonella taylorii*, an unclassified *Bartonella* species, and *B. miyamotoi*.

4 | DISCUSSION

The detection of DOBV RNA in striped field mice at two sites in Austria and the sequence similarity of these sequences to those

from Hungary and Slovakia are in line with results from previous studies in striped field mice from Croatia, Czech Republic, Denmark, Germany, Hungary, Slovakia and Slovenia detecting the human pathogenic DOBV genotype Kurkino (Klempa et al., 2013).

Our study identified the occurrence of multiple *Leptospira* genomospecies and STs in rodents in Austria. The detection of *Leptospira* DNA in a striped field mouse from Austria in this study is in line with detection of *Leptospira* DNA in this species in previous studies in Germany and Hungary (Fischer et al., 2018; Kurucz et al., 2018). The detection of *L. borgpetersenii* ST 146 in a striped field mouse from Austria was a novel finding for this rodent species. *L. borgpetersenii*, ST 146, was previously found in Europe in shrews and bank voles, but not in striped field mice (Fischer et al., 2018). The identification of *L. kirschneri*, ST 110, in bank and common voles in Austria confirmed the association of this *Leptospira* ST with these vole species, previously reported from Germany (Fischer et al., 2018). Common vole-transmitted *L. kirschneri*, ST 110, serovar Grippotyphosa, was the causative agent for a disease cluster in strawberry harvesters in Germany in the year 2007 (Desai et al., 2009).

The detection of *B. taylorii* and *Bartonella* spp. is in line with a previous study in Austria detecting *Bartonella* DNA in other *Apodemus* spp. (Schmidt et al., 2014), but these bacteria were not reported from striped field mice in this country before. *B. taylorii* was also detected in a human patient from Austria (Tobudic et al., 2020), indicating the importance of its detection in rodent reservoirs. *N. mikurensis* was detected in Austrian ticks (Glatz et al., 2014), but thus far not in Austrian rodents except for this work. The detection of *B. miyamotoi* in striped field mouse here accompanies the previous detection of *B. afzelii* in Altenburg and Laa an der Thaya (Schmidt et al., 2014) and indicates that rodents are important reservoirs of these vectorborne pathogens.

In conclusion, the detection of pathogenic DOBV genotype Kurkino, *L. borgpetersenii*, *Bartonella* spp. and *N. mikurensis* in striped field mice underlines the necessity to increase the awareness of physicians in Austria for patients with symptoms of HFRS and leptospirosis as well as vector-borne diseases that suffer from underreporting. The ongoing range expansion of the striped field mouse needs future monitoring studies in a One Health frame by joint efforts of field biologists, veterinarians, clinicians and human virologists.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All relevant guidelines for the use of animals in scientific studies were followed.

DATA AVAILABILITY STATEMENT

Information on the mice investigated here is given in Supporting information Table S1. All new DOBV sequences are deposited at GenBank (for accession numbers, see Figure S1A–C).

ORCID

Kathrin Jeske https://orcid.org/0000-0001-8573-4588
Dusan Kunec https://orcid.org/0000-0001-6697-7621
Jakob Trimpert https://orcid.org/0000-0003-1616-0810
Gerald Heckel https://orcid.org/0000-0002-0162-323X
Rainer G. Ulrich https://orcid.org/0000-0002-5620-1528
Stephan Drewes https://orcid.org/0000-0003-1240-147X

REFERENCES

Aberle, S. (2019). *Starker Anstieg der Puumalavirus Infektionen in Österreich. Virusepidemiologische Information Nr. 11/19-7.* Retrieved from https://www.virologie.meduniwien.ac.at/fileadmin/virologie/files/Epidemiologie/2019/1119.pdf

Blanco, J. R., & Oteo, J. A. (2006). Rickettsiosis in Europe. *Annals of the New York Academy of Sciences*, *1078*, 26–33. https://doi.org/10.1196/annals.1374.003

Bloch, E. M., Kumar, S., & Krause, P. J. (2019). Persistence of *Babesia microti* infection in humans. *Pathogens*, *8*(3), 102. https://doi.org/10.3390/pathogens8030102

Bowen, M. D., Gelbmann, W., Ksiazek, T. G., Nichol, S. T., & Nowotny, N. (1997). Puumala virus and two genetic variants of Tula virus are present in Austrian rodents. *Journal of Medical Virology*, *53*(2), 174–181. https://doi.org/10.1002/(sici)1096-9071(199710)53:2<174:aid-jmv11>3.0.co;2-j

Breitschwerdt, E. B. (2014). Bartonellosis: One health perspectives for an emerging infectious disease. *ILAR Journal*, *55*(1), 46–58. https://doi.org/10.1093/ilar/ilu015

Bundesministerium für Arbeit Soziales Gesundheit und Konsumentenschutz. (2019). Retrieved from https://www.sozialministerium.at/site/Gesundheit/Krankheiten_und_Impfen/Krankheiten/Uebertragbare_Krankheiten/Statistiken_und_Fallzahlen/Jahresstatistiken_meldepflichtiger_Infektionskrankheiten_seit_dem_Jahr_2000

Desai, S., van Treeck, U., Lierz, M., Espelage, W., Zota, L., Sarbu, A., Czerwinski, M., Sadkowska-Todys, M., Avdicová, M., Reetz, J., Luge, E., Guerra, B., Nöckler, K., & Jansen, A. (2009). Resurgence of field fever in a temperate country: An epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. *Clinical Infectious Diseases*, *48*(6), 691–697. https://doi.org/10.1086/597036

Fischer, S., Mayer-Scholl, A., Imholt, C., Spierling, N. G., Heuser, E., Schmidt, S., Reil, D., Rosenfeld, U. M., Jacob, J., Nöckler, K., & Ulrich, R. G. (2018). *Leptospira* genomospecies and sequence type prevalence in small mammal populations in Germany. *Vector-Borne and Zoonotic Diseases*, *18*(4), 188–199. https://doi.org/10.1089/vbz.2017.2140

Glatz, M., Müllegger, R. R., Maurer, F., Fingerle, V., Achermann, Y., Wilske, B., & Bloemberg, G. V. (2014). Detection of candidatus *Neoehrlichia mikurensis*, *Borrelia burgdorferi* sensu lato genospecies and *Anaplasma phagocytophilum* in a tick population from Austria. *Ticks*

Transboundary and Emerging Diseases —WILEY— 5

and Tick-Borne Diseases, *5*(2), 139–144. https://doi.org/10.1016/j.ttbdis.2013.10.006

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I. S., & Harrus, S. (2015). *Bartonella* infection in rodents and their flea ectoparasites: An overview. *Vector-Borne and Zoonotic Diseases*, *15*(1), 27–39. https://doi.org/10.1089/vbz.2014.1606

Hoenigl, M., Wallner, C., Allerberger, F., Schmoll, F., Seeber, K., Wagner, J., Valentin, T., Zollner-Schwetz, I., Flick, H., & Krause, R. (2014). Autochthonous leptospirosis in South-East Austria, 2004–2012. *PLoS One*, *9*(1), e85974. https://doi.org/10.1371/journal.pone.0085974

Ismail, N., Bloch, K. C., & McBride, J. W. (2010). Human ehrlichiosis and anaplasmosis. *Clinics in Laboratory Medicine*, *30*(1), 261–292. https://doi.org/10.1016/j.cll.2009.10.004

Kaya, S., Yilmaz, G., Aydin, M., Aksoy, F., & Koksal, I. (2019). The "MICE" scoring system in differentiating the identical twins leptospirosis and hantavirus infection. *Infection*, *48*, 99–107. https://doi.org/10.1007/s15010-019-01366-7

Klempa, B., Avsic-Zupanc, T., Clement, J., Dzagurova, T. K., Henttonen, H., Heyman, P., Jakab, F., Kruger, D. H., Maes, P., Papa, A., Tkachenko, E. A., Ulrich, R. G., Vapalahti, O., & Vaheri, A. (2013). Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: Definition of genotypes and their characteristics. *Archives of Virology*, *158*(3), 521–529. https://doi.org/10.1007/s00705-012-1514-5

Kurucz, K., Madai, M., Bali, D., Hederics, D., Horvath, G., Kemenesi, G., & Jakab, F. (2018). Parallel survey of two widespread renal syndrome-causing zoonoses: *Leptospira* spp. and hantavirus in urban environment, Hungary. *Vector-Borne and Zoonotic Diseases*, *18*(4), 200–205. https://doi.org/10.1089/vbz.2017.2204

Meerburg, B. G., Singleton, G. R., & Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, *35*(3), 221–270. https://doi.org/10.1080/10408410902989837

Portillo, A., Santibáñez, P., Palomar, A. M., Santibáñez, S., & Oteo, J. A. (2018). 'Candidatus *Neoehrlichia mikurensis*' in Europe. *New Microbes New Infect*, *22*, 30–36. https://doi.org/10.1016/j.nmni.2017.12.011

Richter, D., Klug, B., Spielman, A., & Matuschka, F. R. (2004). Adaptation of diverse lyme disease spirochetes in a natural rodent reservoir host. *Infection and Immunity*, *72*(4), 2442–2444. https://doi.org/10.1128/iai.72.4.2442-2444.2004

Sackl, P., Tiefenbach, M., Tajmel, J., & Spitzenberger, F. (2007). Further spread of the striped field mice *Apodemus agrarius* (PALLAS, 1771) in Austria (Mammalia). *Joannea Zool*, *9*, 5–13 [in German].

Schmidt, S., Essbauer, S. S., Mayer-Scholl, A., Poppert, S., Schmidt-Chanasit, J., Klempa, B., Henning, K., Schares, G., Groschup, M. H., Spitzenberger, F., Richter, D., Heckel, G., & Ulrich, R. G. (2014). Multiple infections of rodents with zoonotic pathogens in Austria. *Vector-Borne and Zoonotic Diseases*, *14*(7), 467–475. https://doi.org/10.1089/vbz.2013.1504

Siński, E., Welc-Falęciak, R., & Zajkowska, J. (2016). *Borrelia miyamotoi*: A human tick-borne relapsing fever spirochete in Europe and its potential impact on public health. *Advances in Medical Sciences*, *61*(2), 255–260. https://doi.org/10.1016/j.advms.2016.03.001

Spitzenberger, F. (1997). Erstnachweis der Brandmaus (*Apodemus agrarius*) für Österreich. *Zeitschrift für Säugetierkunde*, *62*, 250–252.

Spitzenberger, F., & Engelberger, S. (2014). A new look at the dynamic western distribution border of *Apodemus agrarius* in Central Europe (Rodentia: Muridae). *Lynx n.s.*, *45*, 69–79.

Tobudic, S., Burgmann, H., Stanek, G., Winkler, S., Schötta, A. M., Obermüller, M., Markowicz, M., & Lagler, H. (2020). Human *Borrelia miyamotoi* Infection, Austria. *Emerging Infectious Diseases*, *26*(9), 2201–2204. https://doi.org/10.3201/eid2609.191501

Tomassone, L., Berriatua, E., De Sousa, R., Duscher, G. G., Mihalca, A. D., Silaghi, C., Sprong, H., & Zintl, A. (2017). Neglected vector-borne zoonoses in Europe: Into the wild. *Veterinary Parasitology*, *251*, 17–26. https://doi.org/10.1016/j.vetpar.2017.12.018

Vaheri, A., Henttonen, H., Voutilainen, L., Mustonen, J., Sironen, T., & Vapalahti, O. (2013). Hantavirus infections in Europe and their impact on public health. *Reviews in Medical Virology*, *23*(1), 35–49. https://doi.org/10.1002/rmv.1722

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Jeske K, Herzig-Straschil B, Răileanu C et al. Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria. *Transbound Emerg Dis*. 2021;00:1–5. https://doi.org/10.1111/tbed.14015

Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria

<u>**Supporting information**</u>

MATERIAL AND METHODS

Collection of rodents and dissection

Striped field mice were collected during 9th to 12th September 2011 and during 4th to 6th September 2017 by snap trapping in several areas close to Lake Neusiedl (Figure 1A). Permission for trapping was given by the Amt der Burgenländischen Landesregierung in Eisenstadt, Burgenland, Austria (5-N-A1007/444-2011; A4/N.N.AB-10075-5-2017). The carcasses were frozen after trapping, stored at -20°C and transported in dry ice. After thawing at 4°C, the dissection was performed following a standard protocol as described previously (Fischer et al., 2018). In brief, heart, lung, chest cavity fluid (CCF), liver, spleen, and kidney were taken and stored at -20°C until further use. Additional rodents from Lower Austria already tested in a previous study, were used to screen for *Leptospira* DNA enabling characterization on sequence type level (Schmidt et al., 2014) (Figure 1A).

Hantavirus screening and nucleotide sequence determination

RNA from a lentil-sized piece of lung tissue (or liver in case of sample KS12/1827) was extracted with QIAZOL (QIAGEN, Hilden, Germany) and eluted in 100 µl distilled DNase/RNase free water (Thermo Fisher Scientific, Schwerte, Germany) (Schmidt et al., 2014). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using SuperScript™ III One-Step RT-PCR with Platinum Taq-Kit (Invitrogen, Darmstadt, Germany). Dobrava-Belgrade orthohantavirus (DOBV) S segment RT-PCR screening used primer pairs D357M and D1162CM, as well as D113M and D955CM (Table S2). In addition, L segment

1

Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria

Supporting information

MATERIAL AND METHODS

Collection of rodents and dissection

Striped field mice were collected during 9th to 12th September 2011 and during 4th to 6th September 2017 by snap trapping in several areas close to Lake Neusiedl (Figure 1A). Permission for trapping was given by the Amt der Burgenländischen Landesregierung in Eisenstadt, Burgenland, Austria (5-N-A1007/444-2011; A4/N.N.AB-10075-5-2017). The carcasses were frozen after trapping, stored at -20°C and transported in dry ice. After thawing at 4°C, the dissection was performed following a standard protocol as described previously (Fischer et al., 2018). In brief, heart, lung, chest cavity fluid (CCF), liver, spleen, and kidney were taken and stored at -20°C until further use. Additional rodents from Lower Austria already tested in a previous study, were used to screen for *Leptospira* DNA enabling characterization on sequence type level (Schmidt et al., 2014) (Figure 1A).

Hantavirus screening and nucleotide sequence determination

RNA from a lentil-sized piece of lung tissue (or liver in case of sample KS12/1827) was extracted with QIAZOL (QIAGEN, Hilden, Germany) and eluted in 100 µl distilled DNase/RNase free water (Thermo Fisher Scientific, Schwerte, Germany) (Schmidt et al., 2014). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using SuperScript™ III One-Step RT-PCR with Platinum Taq-Kit (Invitrogen, Darmstadt, Germany). Dobrava-Belgrade orthohantavirus (DOBV) S segment RT-PCR screening used primer pairs D357M and D1162CM, as well as D113M and D955CM (Table S2). In addition, L segment

1

sequences from screening-positive animals were generated by one-step L segment RT-PCR using the outer primers Han-LF1 and Han-LR1 (Klempa et al., 2006). For the screening RT-PCR positive sample KS18/1812, the complete S and M segment coding sequences were determined by a primer walking approach (for primers used see Table S2). Dideoxy chain termination sequencing according to Sanger was performed with BigDye Terminator v1.1 Cycle Seq. Kit (Applied Biosystems™, Waltham, MA, USA).

Target enrichment and next generation sequencing of DOBV sequences

Design of target enrichment probes

The DOBV-derived cDNA sequencing libraries were selectively captured with a custom-made target enrichment myBaits array (Arbor Biosciences, Ann Arbor, MI, USA). The array was designed to capture sequences of orthohantaviruses of the Murinae- and Arvicolinae-associated clades from Europe and Asia. The oligonucleotide capture probes were designed based on the available 5,717 nucleotide (nt) sequences of DOBV, Thailand, Seoul, Hantaan, Dabieshan, Puumala and Tula orthohantaviruses deposited in the NCBI GenBank database (January 2020). To collapse the sequence space, the available sequences were clustered based on 95% sequence identity using USEARCH (Edgar, 2010), retaining the longest, representative sequence from each cluster. Simple repeats and low-complexity regions in the sequences were masked with the RepeatMasker (Smit, Hubley, & Green, 2013-2015) and the remaining sequences were used to design 28,579 overlapping 80-nt baits with $3 \times$ tiling density.

Construction of sequencing libraries

Sequencing libraries were constructed using the NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). The input was 100 ng total RNA isolated from lung tissue. RNA was fragmented for 8 minutes and the final cDNA libraries were

2

amplified by 8 PCR cycles to complete adapter ligation and produce sufficient material for subsequent target enrichment.

Target sequence enrichment by hybridization in solution

The enrichment of target DOBV-derived sequences was done by hybridization in solution with pools of biotinylated RNA oligonucleotides. The hybridization-based sequence enrichment (chemistry v3) was performed according to the manufacturer's instructions (Arbor Biosciences, Ann Arbor, MI, USA) allowing 20 hours of bait/target hybridization at 65°C in a single step of enrichment. The enriched cDNA sequencing libraries were amplified with 14 PCR cycles to produce enough DNA material for next-generation sequencing. The enriched cDNA libraries were quantified with the NEBNext Library Quantification Kit (New England Biolabs, Ipswich, MA, USA), pooled to equimolar amounts, and sequenced with a MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA) using paired-end sequencing (2×300 cycles) on a MiSeq sequencer (Illumina, San Diego, CA, USA).

Sequence analysis

The obtained reads were trimmed and assembled against the known complete genome of the DOBV strains GRW/Aa (GenBank JQ026204, JQ026205, JQ026206) and SK/Aa (GenBank GU904039, AY961615, AY961616) using Geneious Prime 2019.2.3 (https://www.geneious.com) with medium sensitivity and allowing 5 iterations.

Hantavirus phylogenetic analysis

ClustalW multiple alignments were constructed using BioEdit v7.2.5 (Hall, 1999). The best fitting substitution model was determined by jModelTest v2.1.6 (Posada, 2008). Phylogenetic trees were constructed according to Maximum Likelihood and Markov chain Monte Carlo

3

algorithms via FasttreeMP v2.1.10 and MrBayes v3.2.6 on CIPRES Science Gateway (Miller et al., 2010, Price et al., 2010, Ronquist et al., 2012). Subsequently a consensus tree was established as bootstrap values were transferred to the Bayesian tree only if branches of both trees were consistent.

Hantavirus serological investigation

DOBV specific IgG-ELISA was performed according a previously published protocol using yeast-expressed DOBV nucleocapsid protein as antigen (Razanskiene et al., 2004, Schlegel et al., 2009). Briefly, 0.2 µg/well of the recombinant protein was coated on 96-well polysorb Nunc-Immuno plates (VWR International GmbH, Hannover, Germany) and incubated for 1 hour with CCF diluted 1:10. After washing, a horse radish peroxidase labelled goat anti-mouse IgG (H+L) (BioRad, Hercules, CA, USA) was used to detect antibodies against the DOBV antigen. Finally, 100 µl of Tetramethylbenzidine (TMB) Peroxidase EIA Substrate Kit (BioRad, Hercules, CA, USA) was added and incubated for 10 min in darkness. The staining was stopped by addition of 100 µl 1M sulfuric acid. Subsequently, optical density was measured with Plate reader Infinite 200 PRO (Tecan, Männedorf, Switzerland) at 450 nm (reference at 620 nm). The monoclonal antibody 5E11 (Kucinskaite-Kodze et al., 2011) was used as positive control; an *Apodemus* spp. CCF tested negative by DOBV-IgG ELISA and DOBV-RT-PCR was applied as negative control. Lower and upper cut-off values were determined according to a previous study (Mertens et al., 2009).

Leptospira **spp. screening**

For *Leptospira* detection a pin-head sized kidney sample was used and DNA extracted with QIAamp DNA Mini Kit (QIAGEN) according to manufacturer´s protocol. Pathogenic *Leptospira* spp. screening followed a *lipl32*-PCR standard protocol (Mayer-Scholl et al., 2011).

4

Genomospecies identification was done by *secY*-PCR and sequence type (ST) was determined by multiple locus sequence typing (MLST) as described before (Boonsilp et al., 2013, Victoria et al., 2008).

DNA extraction for screening of vector-borne pathogens

For detection of vector-borne pathogens, rodent liver and spleen samples were initially homogenized in 2 ml tubes containing one 4 mm sterile metal bead and 200 µl of phosphate buffered solution using a tissue lyser (TissueLyser II; Qiagen, Hilden, Germany) at 30 Hz for 1 min. DNA was extracted using QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions, eluted in 100 µl of elution buffer, and stored at -20°C.

PCR for *Babesia* spp., *Anaplasma/Ehrlichia* spp. and *Bartonella* spp.

DNA extracted from spleen samples was surveyed by PCR for the detection of *Babesia* spp., targeting a fragment of 422-440 base pairs (bp) of 18S rRNA gene (Hilpertshauser, Deplazes, Schnyder, Gern, & Mathis, 2006), *Anaplasma/Ehrlichia* spp. using specific primers for a 345 bp fragment of 16S rRNA gene (Parola et al., 2000) and *Bartonella* spp. by amplifying a fragment of 420-780 bp of 16S-23S rRNA (Schorn, Pfister, Reulen, Mahling, & Silaghi, 2011). The reactions were performed using GoTaq® Flexi DNA Polymerase kit (Promega, Walldorf, Germany). The reaction mix included 3 µl $MgCl_2$ solution (25mM), 0.5 µl dNTPs (10mM), 0.125 µl GoTaq® Flexi DNA polymerase (5 u/µl), 1µl of each primer (10 µM), 9.375 µl molecular grade water and 5 µl of the tested DNA. Cycling conditions were as follows: initial denaturation for 2 min at 95°C was followed by 35 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 61°C for *Babesia* spp., 53°C for *Anaplasma/Ehrlichia* spp. and 66°C for *Bartonella* spp., elongation (60 sec for *Bartonella* spp. and 30 sec for *Anaplasma/Ehrlichia* spp. and *Babesia* spp.) at 72°C, and a final extension at 72°C for 5 min. Each reaction included a negative control (water) and positive controls (DNA from ticks for *Anaplasma/Ehrlichia* spp.

5

and *Babesia* spp., DNA from fleas for *Bartonella* spp.). Primers used for the PCR assays described here are given in Table S3.

Nested PCR for *Borrelia* spp.

For the detection of *Borrelia* species in spleen samples, nested PCR was done using specific primers for the 16S-23S intergenic spacer (16S-23S IGS; see Table S3) as previously described (Bunikis et al., 2004). The reaction was done using GoTaq® Flexi DNA Polymerase kit (Promega, Walldorf, Germany) targeting in the first PCR a fragment of approximately 1007 bp (size varies with the *Borrelia* species) and a fragment of 388–685 bp in the second PCR. Negative (water) and positive controls (DNA from ticks) were used with each run.

Real time PCR for the detection of *Anaplasma phagocytophilum* and *Neoehrlichia mikurensis*

Real time PCR (qPCR) was performed using specific primers and probes (see Table S3) in order to identify a 77 bp fragment of *A. phagocytophilum msp2* gene (Courtney, Kostelnik, Zeidner, & Massung, 2004), and a 99 bp fragment of *N. mikurensis groEL* gene (Jahfari et al., 2012, Silaghi, Woll, Mahling, Pfister, & Pfeffer, 2012), respectively. Taqman qPCR reactions were performed in a final volume of 25 μl using the iTaq Universal Probes Supermix (Bio-Rad Laboratories, Feldkirchen, Germany). The qPCR program included an initial denaturation step at 95°C for 10 min followed by 45 cycles of amplification at 95°C for 45 sec and 60°C for 60 sec and final cooling. The reactions included a negative control (water) and positive controls (DNA from ticks).

Molecular detection of *Rickettsia* spp. in liver samples

For the identification of *Rickettsia* species in liver samples of rodents, a PCR reaction was performed to amplify a 381 bp fragment of *gltA* gene using specific forward and reverse primers

6

(Regnery, Spruill, & Plikaytis, 1991; see Table S3). The amplification was done using GoTaq® Flexi DNA Polymerase kit (Promega, Walldorf, Germany) and the conditions were identical as the ones used in the PCRs for spleen samples with the exception of the annealing temperature that was set at 56°C for 30 sec. Each reaction contained a negative (water) and positive (DNA from field ticks) control.

Sequencing of PCR products

The products from the PCR and nested PCR reactions were separated on a 1.5% agarose gel stained with Roti®-GelStain Red (Carl Roth GmbH, Karlsruhe, Germany) and visualised with ChemiDoc™ MMP Imaging system (Bio-Rad Laboratories, Feldkirchen, Germany). The positive amplicons were purified using the NucleoSEQ® kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instructions. The sequencing PCR reactions were performed in a total volume of 10 µl: 1 µl of 5x Sequence buffer, 2 µl Big Dye Ready Reaction Mix (Thermo Fischer, Darmstadt, Germany), 1 µl of reverse primer (10 µM), 5 µl of molecular grade water and 1 µl of the purified PCR product. The thermal profile included a denaturation step at 96°C for 1 min, followed by 25 cycles of denaturation at 96°C for 10 sec, annealing at the specific annealing temperature for each primer for 5 sec, elongation at 60°C (duration varying with the length of DNA fragments). Further, the PCR products were purified using the NucleoSEQ kit (Macherey-Nagel, Düren, Germany). Fifteen microliters of each purified product were mixed with 15 µl of highly deionized (Hi-Di) formamide in a 1.5 ml tube and sequenced on an ABI PRISM® 3130 sequencer. Following sequencing, the obtained sequences were viewed and edited using Geneious 11.1.5 (https://www.geneious.com) and then compared with data available in the GenBank database using BLASTn.

7

Statistics

The 95% confidence interval for prevalences was determined using the exact binomial two-sided test for 95% confidence interval with software R (version 3.6.1).

8

Table S1. Results of pathogen screening of striped field mice (*Apodemus agrarius*).

Sample categories	Number of animals	DOBV-ELISA	DOBV-RT-PCR	lipl32-PCR	Babesia PCR	Anaplasma/ Ehrlichia PCR	Anaplasma phagocytophilum PCR	Bartonella PCR	Borrelia PCR	Neoehrlichia mikurensis PCR	Coinfection
All mice	35	2/35 (5.7%, CI 0.7-19.2%)	2/35 (5.7%, CI 0.7-19.2%)	1/35 (2.9%, CI 0.1-14.9%)	0/32	0/32	0/32	4†‡/32 (12.5%, CI 3.5-29.0%)	1§/32 (3.1%, CI 0.8-20.8%)	2/32 (6.3%, CI 0.8-20.8%)	1#/32 (3.1%, CI 0.8-20.8%)
Sex											
Male	19	1/19	1/19	0/19	0/17	0/17	0/17	2†‡/17	1§/17	2/17	1#/17
Female	16	1/16	1/16	1/16	0/15	0/15	0/15	2†‡/15	0/15	0/15	0/15
Trapping area (sites)											
A (4,6,9,10,12)	14	1/14	1/14	0/14	0/13	0/13	0/13	1†/13	0/13	1/13	0/13
B (2,3,5)	6	1/6	1/6	1/6	0/6	0/6	0/6	1†/6	1§/6	0/6	0/6
C (1,7,8,11,13)	15	0/15	0/15	0/15	0/13	0/13	0/13	2‡/13	0/13	1/13	1#/13
Weight											
<15g	7	0/7	0/7	0/7	0/6	0/6	0/6	0/6	0/6	0/6	0/6
15-20g	19	0/19	0/19	1/19	0/18	0/18	0/18	3/18	0/18	0/18	0/18
>20g	9	2/9	2/9	0/9	0/8	0/8	0/8	1/8	1§/8	2/8	1#/8

Abbreviations: CI confidence interval
†*Bartonella taylorii*, ‡*Bartonella* sp., §*Borrelia miyamotoi*
#Coinfection with *Bartonella* sp. and *Neoehrlichia mikurensis*; no other coinfections were detected.

9

138

Table S2. Primers used for Dobrava-Belgrade orthohantavirus screening and additional primers used for primer-walking-based determination of complete coding sequences of S and M segments.

| Primer	Sequence 5´→3´	Reference
D357M	GACATTGATGAACCAACAGG	Klempa, 2004, modified
D1162CM	AGYTGRATHCCCATRGAYTGT	Klempa, 2004, modified
D113M	GATGCAGARAARCARTATGARAA	Klempa, 2004, modified
D955CM	ACCCARATTGATGAYGGTGA	Klempa, 2004, modified
HanLF1	ATGTAYGTBAGTGCWGATGC	Klempa et al., 2006
HanLR1	AACCADTCWGTYCCRTCATC	Klempa et al., 2006
DS3F	GTAGTAGRCTCCCTAAARAGCAC	this study
DS720R	CKTTCTGTCCAGTTCTTWGCAAG	this study
DS970F	ACCTGATAGRTGCCCCCCYA	this study
DS1455R	ACCCGTCCCCTAGTGCAAAT	this study
DM3F	GTAGTAGRCTCCGCAAGAAATAG	this study
DM956R	AGTRAATRVCATATTTGCTGCATTTKC	this study
DM644F	GAYATWGCAAGTGTVAGYATTGTYTG	this study
DM1280R	TCTGCAGCCCTGAAYCKRTT	this study
DM803F	CRGTRGTTGAGGGTGCATARAC	this study
DM1451F	GCAATTGAAYTRTGTGTRCCHGG	this study
DM2010R	CRACACCATGGTGCATTRTCATTCC	this study
DM1970F	TGGGCTGHAGTGCATCAGA	this study
DM3200R	AGCACTTGCTTGAAGCCCTTG	this study
DM2621F	GAYTGGTGYACAACAACRTGYC	this study
DM3615R	GTAKGCTCCGCAAGATATAGAAAYAC	this study

10

Table S3. Oligonucleotides used for tick-borne pathogen detection in spleen and liver samples.

| Pathogen	Target	Primer/Probe	Sequence (5'→3')	Reference
Babesia spp.	*18S rRNA*	BabsppF1	GTTTCTGMCCCATCAGCTTGAC	Hilpertshauser et al., 2006
		BabsppR	CAAGACAAAAGTCTGCTTGAAAC	
Anaplasma/Ehrlichia spp.	*16S rRNA*	EHR16SD	GGTACCYACAGAAGAAGTCC	Parola et al., 2000
		EHR16SR	TAGCACTCATCGTTTACAGC	
Bartonella spp.	*16s-23S rRNA*	BA325s	CTTCAGATGATGATCCCAAGCCTTCTGGCG	Schorn et al., 2011
		BA1100as	GAACCGACGACCCCCTGCTTGCAAAGCA	
Borrelia spp.	*16S-23S rRNA*	Bospp-IGS-F	GTATGTTTAGTGAGGGGGGTG	Bunikis et al., 2004
		Bospp-IGS-R	GGATCATAGCTCAGGTGGTTAG	
		Bospp-IGS-Fi	AGGGGGGTGAAGTCGTAACAAG	
		Bospp-IGS-Ri	GTCTGATAAACCTGAGGTCGGA	
A. phagocytophilum	*Msp2*	ApMSP2f	ATGGAAGGTAGTGTTGGTTATGGTATT	Courtney et al., 2004
		ApMSP2r	TTGGTCTTGAAGCGCTCGTA	
		ApMSP2p	TGGTGCCAGGGTTGAGCTTGAGATTG	
N. mikurensis	*groEL*	NMikGroEL F2	CCTTGAAAATATAGCAAGATCAGGTAG	Jahfari et al., 2012, Silaghi et al., 2012
		NMikGroEL rev1	CCACCACGTAACTTATTTAGCACTAAAG	
		NMikGroEL rev2	CCACCACGTAACTTATTTAGTACTAAAG	
		NMikGroEL_P2a	CCTCTACTAATTATTGCTGAAGATGTAGAAGGTGAAGC	
Rickettsia spp.	*gltA*	Rsfg877	GGGGGCCTGCTCACGGCGG	Regnery et al., 1991
		Rfsg1258	ATTGCAAAAAGTACAGTGAACA	
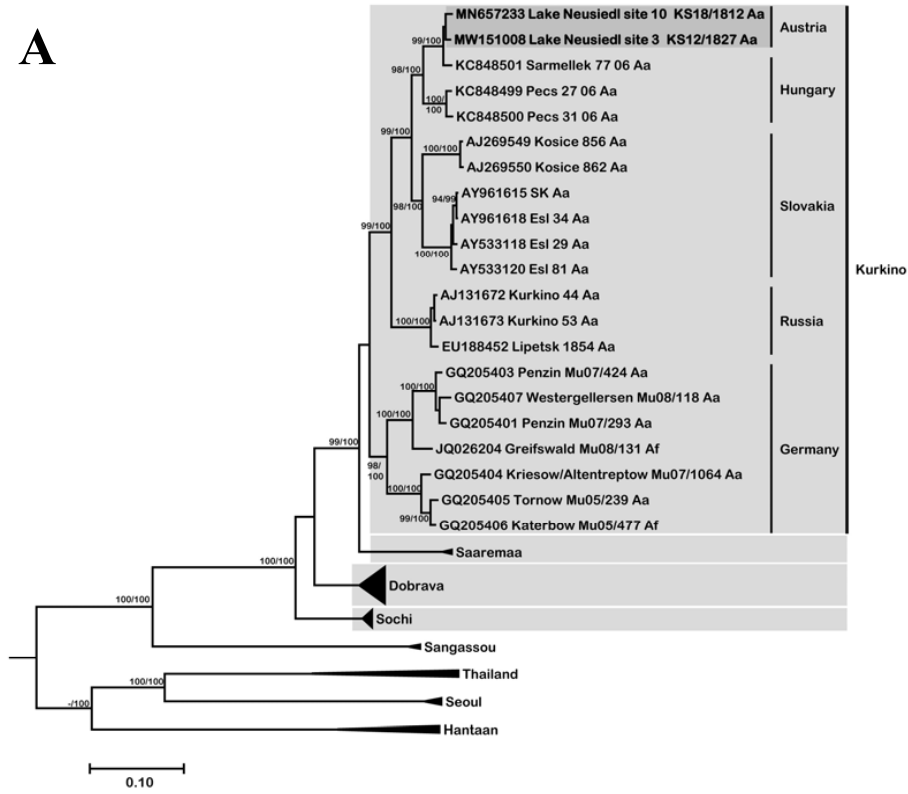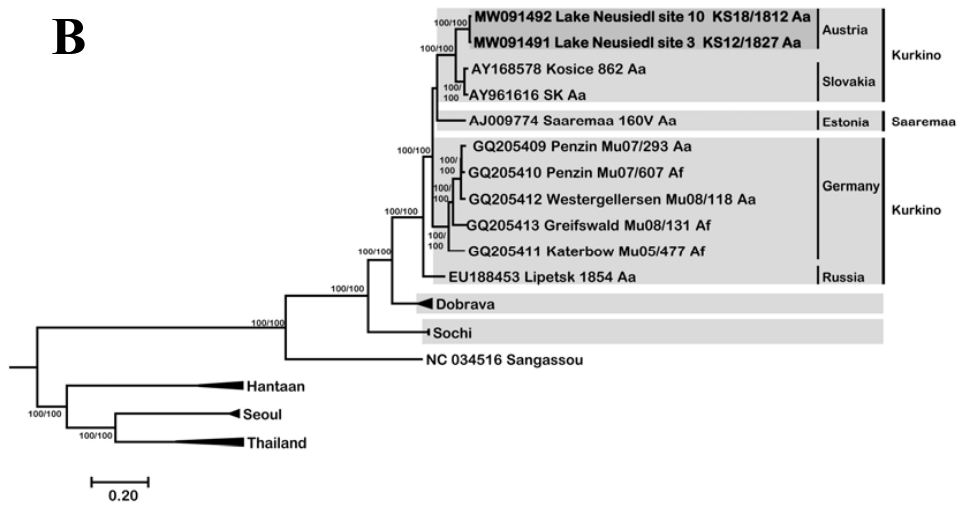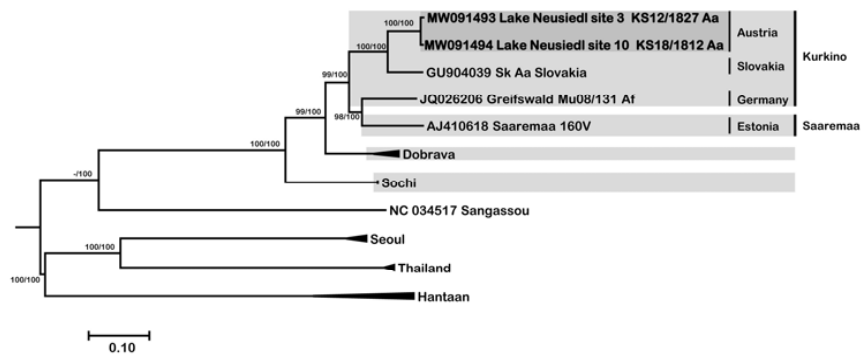
FIGURE S1

Consensus phylogenetic trees of Dobrava-Belgrade orthohantavirus (DOBV) based on complete nucleocapsid protein coding S segment sequences **(A)**, complete glycoprotein precursor coding M segment sequences **(B)**, and complete RNA-dependent RNA polymerase coding L segment sequences **(C)**. Classification of DOBV genotypes is given as previously suggested (Klempa et al., 2013). General Time Reversible (GTR) substitution model with invariant sites and a gamma distributed shape parameter was used for tree calculation. The consensus tree is based on Bayesian analyses with 1,000,000 generations and a burn-in phase of 25%. The condensed DOBV clade of genotype Dobrava comprise the following sequences (**S**: AJ009775, AJ410615, AJ410619, AY168576, EU188452, GU904029, KC676589, KC676590, KC676591, KC676595, KC676597, KC676599, KC676602, KC848494-KC848498, L41916; **M:** AJ410616, AY168577, GU904035, L33685; **L:** AJ410617, GU904042, KT885041), whereas the condensed DOBV clade of genotype Sochi consist of (**S:** EU188449, EU188450, JF920151, JF920152, KP878311- KP878313, MH251334; **M:** EU188450, JF920149, MH251335; **L:** JF920148; MH251336). Sangassou (JQ082303, JQ082300), Hantaan (AF288646, EU02220, FJ753396, GU329991, JQ665905, KC844227), Thailand (AM397664, KC490915, KC490916), and Seoul orthohantavirus strains (GU592951, JX879769, KC626089) were used as outgroup for **(A)**. Sangassou (NC_034516), Hantaan (DQ371905, KJ857334, KP970561), Thailand (L08756, KC490919, KC490921), and Seoul orthohantavirus strains (JX879768, KM948593, S47716) were used as outgroup for **(B)**. Sangassou (NC_034517), Hantaan (DQ371906, KJ857317, KP896308), Thailand (KC490922-KC490924), and Seoul orthohantavirus (JX879770, KM948594, X56492) were used as outgroup for **(C)**.

References

Bunikis, J. Garpmo, U. Tsao, J. Berglund, J. Fish, D. Barbour, AG. (2004). Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. *Microbiology*, 150 (Pt 6), 1741-1755. https://doi.org/10.1099/mic.0.26944-0

Boonsilp, S., Thaipadungpanit, J., Amornchai, P., Wuthiekanun, V., Bailey, M. S., Holden, M. T., . . . Peacock, S. J. (2013). A single multilocus sequence typing (MLST) scheme for seven pathogenic Leptospira species. *PLoS Negl Trop Dis, 7*(1), e1954. https://doi.org/10.1371/journal.pntd.0001954

Courtney, JW. Kostelnik, LM. Zeidner, NS. Massung, RF. (2004). Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *J Clin Microbiol*, 42(7), 3164-3168. https://doi.org/10.1128/JCM.42.7.3164-3168.2004

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19), 2460-2461. https://doi.org/10.1093/bioinformatics/btq461

Fischer, S., Mayer-Scholl, A., Imholt, C., Spierling, N. G., Heuser, E., Schmidt, S., . . . Ulrich, R. G. (2018). *Leptospira* Genomospecies and Sequence Type Prevalence in Small Mammal Populations in Germany. *Vector Borne Zoonotic Dis, 18*(4), 188-199. https://doi.org/10.1089/vbz.2017.2140

Hall, T. A. (1999). *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.* Paper presented at the Nucleic acids symposium series.

Hilpertshauser, H. Deplazes, P. Schnyder, M. Gern, L. Mathis, A. (2006). *Babesia* spp. identified by PCR in ticks collected from domestic and wild ruminants in southern Switzerland. *Appl Environ Microbiol,* 72 (10) 6503-6507. https://doi.org/10.1128/AEM.00823-06

Jahfari S., Fonville M., Hengeveld P., Reusken C., Scholte EJ., Takken W., . . . Sprong, H (2012). Prevalence of *Neoehrlichia mikurensis* in ticks and rodents from North-west Europe. *Parasit Vectors,* 5,74, https://doi.org/10.1186/1756-3305-5-74

Klempa, B. (2004). *Dobrava and Tula hantaviruses from Central Europe: molecular evolution and pathogenic relevance.* PhD thesis, Humboldt-Universität zu Berlin, pp. 1-125

Klempa, B., Avsic-Zupanc, T., Clement, J., Dzagurova, T. K., Henttonen, H., Heyman, P., . . . Vaheri, A. (2013). Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: definition of genotypes and their characteristics. *Arch Virol, 158*(3), 521-529. https://doi.org/10.1007/s00705-012-1514-5

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., . . . Krüger, D. H. (2006). Hantavirus in African wood mouse, Guinea. *Emerg Infect Dis, 12*(5), 838-840. https://doi.org/10.3201/eid1205.051487

Kucinskaite-Kodze, I., Petraityte-Burneikiene, R., Zvirbliene, A., Hjelle, B., Medina, R. A., Gedvilaite, A., . . . Ulrich, R. G. (2011). Characterization of monoclonal antibodies against hantavirus nucleocapsid protein and their use for immunohistochemistry on rodent and human samples. *Arch Virol, 156*(3), 443-456. https://doi.org/10.1007/s00705-010-0879-6

Mayer-Scholl, A., Draeger, A., Luge, E., Ulrich, R., & Nockler, K. (2011). Comparison of two PCR systems for the rapid detection of *Leptospira* spp. from kidney tissue. *Curr Microbiol, 62*(4), 1104-1106. https://doi.org/10.1007/s00284-010-9829-5

Mertens, M., Wolfel, R., Ullrich, K., Yoshimatsu, K., Blumhardt, J., Romer, I., . . . Ulrich, R. G. (2009). Seroepidemiological study in a Puumala virus outbreak area in South-East

Germany. *Med Microbiol Immunol, 198*(2), 83-91. https://doi.org/10.1007/s00430-009-0106-9

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Paper presented at the 2010 gateway computing environments workshop (GCE), New Orleans, LA, USA. https://doi.org/10.1109/GCE.2010.5676129

Parola, P. Roux ,V. Camicas, JL. Baradji, I. Brouqui, P. Raoult, D. (2000). Detection of ehrlichiae in African ticks by polymerase chain reaction. *Trans R Soc Trop Med Hyg*, 94 (6), 707-708. https://doi.org/10.1016/s0035-9203(00)90243-8

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Mol Biol Evol, 25*(7), 1253-1256. https://doi.org/10.1093/molbev/msn083

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One, 5*(3), e9490. https://doi.org/10.1371/journal.pone.0009490

Razanskiene, A., Schmidt, J., Geldmacher, A., Ritzi, A., Niedrig, M., Lundkvist, Å., . . . Ulrich, R. (2004). High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast *Saccharomyces cerevisiae*. *J Biotechnol, 111*(3), 319-333. https://doi.org/10.1016/j.jbiotec.2004.04.010

Regnery, RL. Spruill, CL. Plikaytis, BD. (1991). Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol, 173 (5), 1576-1589. https://doi.org/10.1128/jb.173.5.1576-1589.1991

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., . . . Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol, 61*(3), 539-542. https://doi.org/10.1093/sysbio/sys029

Schlegel, M., Klempa, B., Auste, B., Bemmann, M., Schmidt-Chanasit, J., Büchner, T., . . . Ulrich, R. G. (2009). Dobrava-Belgrade virus spillover infections, Germany. *Emerg Infect Dis, 15*(12), 2017-2020. https://doi.org/10.3201/eid1512.090923

Schmidt, S., Essbauer, S. S., Mayer-Scholl, A., Poppert, S., Schmidt-Chanasit, J., Klempa, B., . . . Ulrich, R. G. (2014). Multiple infections of rodents with zoonotic pathogens in Austria. *Vector Borne Zoonotic Dis, 14*(7), 467-475. https://doi.org/10.1089/vbz.2013.1504

Smit, A. F. A., Hubley, R., & Green, P. (2013-2015). RepeatMasker Open-4.0. Retrieved from http://www.repeatmasker.org

Schorn, S. Pfister, K. Reulen, H. Mahling, M. Silaghi, C. (2011). Occurrence of *Babesia* spp., *Rickettsia* spp. and *Bartonella* spp. in *Ixodes ricinus* in Bavarian public parks, Germany. *Parasit Vectors,* 4, 135. https://doi.org/10.1186/1756-3305-4-135

Silaghi, C. Woll, D. Mahling, M. Pfister, K. Pfeffer, M. (2012). *Candidatus* Neoehrlichia mikurensis in rodents in an area with sympatric existence of the hard ticks *Ixodes ricinus* and *Dermacentor reticulatus*, Germany. *Parasit Vectors,* 5, 285. https://doi.org/10.1186/1756-3305-5-285

Victoria, B., Ahmed, A., Zuerner, R. L., Ahmed, N., Bulach, D. M., Quinteiro, J., & Hartskeerl, R. A. (2008). Conservation of the S10-spc-alpha locus within otherwise highly plastic genomes provides phylogenetic insight into the genus Leptospira. *PLoS One, 3*(7), e2752. https://doi.org/10.1371/journal.pone.0002752

4 Results and Discussion

4.1 Distribution of novel and known rodent-borne hantaviruses in Europe

4.1.1 First detection of strains of putative Tatenale orthohantavirus in continental Europe

In Germany, Traemmersee hantavirus (TRAV) was reported in one field vole (PAPER I). A related hantavirus, Rusne hantavirus (RUSV), was detected in root voles from Lithuania (PAPER II). These viruses seem to be related to Tatenale hantavirus (TATV) in England based initially on the available short sequence fragments of S and L. Later on, when complete coding sequences of TATV were available, pairwise evolutionary distance (PED) analysis (see 1.4.1.) proved that TATV, TRAV and RUSV belong to the same putative orthohantavirus species (PAPER II, Table S5, Chappell et al. 2020).

TATV is a new hantavirus that was discovered a few years ago in field voles in England (Pounder et al. 2013; Thomason et al. 2017), the only *Microtus* species on the British island (Pardiñas et al. 2017). TATV was reported at different years and locations, for example TATV from Tattenhall, close to Chester in Northwestern England, and TATV strain Kielder from Kielder forest in Northern England (Pounder et al. 2013; Thomason et al. 2017). Geographic clustering of TATV sequences from both trapping locations into phylogenetic trees (PAPER I, Figure 1 and PAPER II, Figure S2, Thomason et al. 2017, Chappell et al., 2020) may be due to geographic distance or due to adaption of the virus to the different field vole lineages. The field voles from Tattenhall belong to the Western lineage, while those from Kielder belong to the Northern Britain lineage (Herman et al. 2014; Pounder et al. 2013; Thomason et al. 2017). This observation might be explained by a coevolution scenario that was previously reported for TULV, where different virus lineages were reported to be strictly specific to common vole lineages (Saxenhofer et al. 2019).

In Germany and Lithuania (PAPER I, Table 1 and PAPER II, Table 1), in contrast to England (Pounder et al. 2013; Thomason et al. 2017), the respective hantavirus was only detected at one of several sites. Of note regarding RUSV, the root vole seems to be a relatively new species to Lithuania. First mentioned in 1964 (Ivanauskas et al. 1964), this vole species increased its distribution range from southwestern Lithuania (including the Nemunas River Delta with Rusné), where root voles are present with the highest density in the present, to northeastern

Lithuania, based on findings from 2008 and recent trapping results (PAPER II; Balčiauskas et al. 2010; Balčiauskas et al. 2012). High density of the reservoir population is essential for maintaining hantaviruses in the population, as reported for PUUV (Laenen et al. 2019b). Perhaps the root vole density in most parts of Lithuania, except for the Nemunas River Delta with Rusné, is insufficient to maintain RUSV, explaining its limited geographic range (PAPER II). The reason for the limited detection of TRAV is unknown (PAPER I). In combination with other studies testing field voles and other *Microtus* spp. in Germany, it is surprising that TRAV was never detected before (Schmidt-Chanasit et al. 2010; Schmidt et al. 2016).

In Germany, TRAV was detected in 2008 at the trapping site Trämmersee with a prevalence of 12.5% and in general with a prevalence of 0.8% in field voles (PAPER I). In contrast to the low detection rate of TRAV, RUSV was detected in Rusné with a prevalence ranging from 12.5% up to 14.3% in the years of 2015 to 2017, except for 2018 (PAPER II). The detected prevalence for TATV in field voles in England was higher than for TRAV and comparable to RUSV. It ranged depending on area from 8.3% up to 25% (Chappell et al. 2020; Pounder et al. 2013; Thomason et al. 2017), except for areas with a low number of captured field voles (Thomason et al. 2017).

For a certain species to be recognized as a reservoir of a pathogen, criteria such as multiple detection of the pathogen over time need to be fulfilled (see 1.1.2). Field voles in England may act as a reservoir species for TATV, as TATV was detected in field voles at several locations in different years (Pounder et al. 2013; Thomason et al. 2017). TRAV had a single detection in a field vole from Germany (PAPER I). While TATV and TRAV were both detected in field voles, RUSV was only detected in root voles, though field voles were present at the positive sampling site and other sites in Lithuania along with common voles (PAPER II). Likewise to TRAV, RUSV was only detected in one location, but unlike TRAV, it was detected in several years, making it likely that root voles act as a reservoir of RUSV in the Nemunas River Delta in Lithuania (PAPER I and PAPER II). Another explanation might be based on the existence of an ancestral hantavirus. Based on the detection of TATV and TRAV only in field voles, it seems likely that the new hantavirus was already present in the Balkan refugium of the field voles. From there, the field vole re-colonialized Central Europe after the Last Glacial Maximum (LGM) (Fletcher et al. 2019), spread north to Germany, Eastern Europe and France, where the field vole split over time into the different field vole lineages (Jaarola and Searle 2002). England, in which TATV was detected, was colonized by the Western field vole lineage after the LGM 14,700 years before present (BP) and before the land bridge connecting England with

continental Europe disappeared 8,400 years BP with the rise of the sea level. Afterwards, the Western field vole lineage split further with the colonization of the north into a Northern Britain lineage (Herman and Searle 2011).

However, if the root vole population was only recently established in Lithuania and had probably migrated from the southwest (Balčiauskas et al. 2010), where did the RUSV detected only in root voles (PAPER II) originated from? Poland, lying between Germany and Lithuania, is inhabited by both root voles and field voles (see 1.2.3.). Eastern Poland possesses optimal root vole habitats, resulting in a high-density population, and the Central root vole lineage, which is present in Lithuania, can be detected there (Dabrowski et al. 2013). In Białowieża forest in Eastern Poland, root voles have even been abundantly present since the postglacial period (Dabrowski et al., 2013). This might indicate that root vole populations in Poland could sustain RUSV or a related hantavirus. In fact, a Fusong-related virus has been described in Eastern Poland in one field vole, but the sequence was not uploaded (Wójcik-Fatla et al. 2013). The findings of a sequence similar to Fusong orthohantavirus (as was observed using the nucleotide Basic Local Alignment Search Tool (nBLAST) to determine the hantavirus species of the first TRAV sequences and TRAV clustered close to Fusong orthohantavirus in phylogenetic trees (PAPER I, Fig. S3 A-F)) in a field vole, highlight the possibility that TRAV and/or RUSV are present in Poland. However, it is unclear in which species this hantavirus might be maintained in Poland, as the study monitored only 60 small mammals and subjects were found dead at two sites. The samples included one negative-tested common vole and one positive-tested field vole but did not include any root voles (Wójcik-Fatla et al. 2013).

Arviolinae-associated hantaviruses are believed to originate in Asia (Souza et al., 2014). Therefore, it seems likely that TATV split from a precursor virus within a precursor Arvicolinae species, which was probably a *Microtus* species and split according to the radiation and evolution of this genus. The evolution and radiation of the genus *Microtus* was proposed to be in Central Asia, with the root vole splitting earlier from the common ancestor and common voles and later from the field voles (Barbosa et al. 2018). The root vole also later colonized Europe as well as North America. If the TATV or a related ancestral virus was present in root voles and not field voles during that time, a similar virus should be detectable in specimens from Asia and/or North America, given that the virus is not extinct. The only detection of a hantavirus before the one root vole in Russia, which possibly was a spillover event, was that of the Fusong strain of Vladivostok orthohantavirus that is usually harbored by the reed vole (*Microtus fortis*) (Plyusnina et al. 2008b). A host switch event could have later occurred in a

147

contact zone between field voles and root voles. The frequency of *Microtus* vole-associated viruses to switch hosts or cause spillovers (see 4.1.2., Plyusnina et al. 2008b), suggest that close similarity of the respective hantavirus target receptors and/or similar immune response upon infection, caused by relatively fast species radiation, might be the reason (Barbosa et al. 2018). A certain difference of RUSV from the field vole-associated viruses seems likely to have been the determining factor for the recent host switch event. In RUSV only, there is an insertion in the open-reading frame of the S segment encoding for the non-structural protein that has remained stable over the years. A similar but different insertion was only found in KHAV (PAPER II), which was detected in reed vole (Plyusnina et al. 2008b). The reed vole belongs to the same genus *Alexandromys* as the root vole (Barbosa et al. 2018), which perhaps indicates an evolutionary adaption. This protein is only expressed in Arvicolinae-, Neotominae-, and Sigmodontinae-associated hantaviruses and was assumed to act as an interferon antagonist (Jääskelainen et al. 2007; Jääskeläinen et al. 2008). Whether this insertion is an adaption to the innate immunity in root voles lacking in field voles cannot be answered at this time, but it remains a question for further research.

It is currently unknown whether TATV is zoonotic. Tests with the new antigen resulted in detection of antibodies against TATV strain Rusne in four of eight RT-PCR positive-tested root voles. In Western blot and ELISA, a high cross-reactivity to PUUV and ANDV/SNV was observed (PAPER II, Table 2).

4.1.2 Tula orthohantavirus in Europe

TULV is known as a widespread hantavirus in Eurasia, with genetic detection in countries in the West, from France (Schmidt et al. 2016), to the East, in China (Guo et al. 2019). The detection of TULV in Germany (PAPER I and PAPER IV) is in line with several earlier studies in which this hantavirus was detected (Klempa et al. 2003a; Mertens et al. 2011a; Saxenhofer et al. 2019; Schlegel et al. 2012; Schmidt-Chanasit et al. 2010; Schmidt et al. 2016). Surprisingly, no TULV was detected in common voles from Spain (Paper III). If TULV was indeed present in common voles in this country, it is unlikely that it would have gone unnoticed with the two RT-PCRs used, targeting the S and the L segment, respectively. The S segment RT-PCR is not only able to detect TULV from different lineages (PAPER I and PAPER IV, Schmidt et al. 2016), but also other Arvicolinae-associated hantaviruses such as PUUV (PAPER IV, Drewes et al. 2017a; Essbauer et al. 2006), TRAV and RUSV (PAPER I and PAPER II). The L segment RT-PCR targeting a highly conserved region on the RNA-dependent

RNA polymerase is even able to detect several hantavirus species, ranging from the genus *Orthohantavirus*, such as TULV and Muridae-associated hantaviruses (Sangassou orthohantavirus), to the genus *Loanvirus* with Brno loanvirus (Guo et al. 2019; Klempa et al. 2006; Straková et al. 2017). The sampled common voles and Lusitanian pine voles were captured in different years from different sites in Spain. During the capturing period, a common vole outbreak occurred. This resulted in high prevalence of even the pathogen *Francisella tularensis*, which is usually detected in lower prevalence (Jeske et al. 2019a; Kaysser et al. 2008; Luque-Larena et al. 2015). Therefore, it is highly unlikely, due to the sample size, number of different trapping years and areas and the two RT-PCRs used, that TULV would fail to be detected in common voles in Spain. It is more likely that TULV can be assumed to be absent from the common vole population on the Iberian Peninsula.

The Western lineage of the common vole is present in Spain (Heckel et al. 2005). Some authors specify this lineage further as Iberian clade, or Western-South lineage (García et al. 2020). The Western common vole lineage in France was found to be associated with the Central South TULV lineage, but only in a region close to the Central common vole lineage, which is the main reservoir of this TULV lineage (Saxenhofer et al. 2019; Schmidt et al. 2016). The Iberian Peninsula was colonized between 23,000 and 21,000 years ago by the Western common vole lineage (García et al. 2020). It is assumed that the common vole survived during the LGM 23,000 to 18,000 years BP by inhabiting refugia such as the Mediterranean and the Balkan refugium, which, in combination with the cold-persistence of this species, enabled subsequent survival of this vole in suitable ice-free habitats even outside the refugia (Heckel et al. 2005). The Western lineage of the common vole was split during the LGM by the Pyrenean glaciers. It is likely that common voles survived the LGM on the Iberian Peninsula (south from the Pyrenees in a refugium) and north from the Pyrenees in a refugium and re-colonized respectively Spain and France (García et al. 2020; Heckel et al. 2005). Nonetheless, the common vole on the Iberian Peninsula was thereafter geographically isolated from the rest of the European common vole population by the Pyrenees and/or the Ebro River (García et al. 2020). TULV may have only gone extinct in the isolated Iberian population. It is currently unknown if TULV is also extinct in the rest of the Western common vole population, excluding the contact zone to the Central common vole lineages in France mentioned above.

Consistent with other publications (Saxenhofer et al. 2019; Schmidt et al. 2016), the Central North TULV clade was detected in Germany in Thuringia and Brandenburg (PAPER I and PAPER IV). The detected TULV prevalence of 13.8% in common voles, which ranged between

0% and 33.8% among trapping sites, in Germany (PAPER III) is consistent with the general detection rate in Germany and other countries (Table 3), in contrast to the low prevalence of 7.7% in common voles in Brandenburg (PAPER I).

A difference in TULV-prevalence in common voles between different studies, even in the same country, has been observed (PAPER I, PAPER III, PAPER IV, and Table 3). The reason for this is that the TULV prevalence can be impacted by several factors that were also described for other hantaviruses and their rodent hosts. Whereas knowledge about the common vole-TULV relationship, except for simple screening studies, is rare, the bank vole-PUUV relationship is the best studied host-hantavirus system in Europe. To a certain point, discovered features of this relationship can be presumed for the relationship between common voles and TULV, as the hantaviruses are closely related and the hosts share a geographic distribution and are at least members of the subfamily Arvicolinae. TULV was detected with the highest prevalence in spring, followed by fall (PAPER IV). Common voles trapped during the summer months showed a lower prevalence (PAPER I and PAPER IV). This can be explained by European hantaviruses showing a strong seasonality, with the highest prevalence being detected in spring, followed by fall, and the lowest prevalence in summer (PAPER III; Deter et al. 2008; Khalil et al. 2016; Niklasson et al. 1995), although a single study reported lower prevalence in spring (Kurucz et al. 2018). The observed seasonality is an effect of already infected individuals surviving the winter, resulting in high prevalence in spring, which decreases in summer due to the birth of offspring that can be protected by maternal antibodies up to two months if they are the offspring of infected mothers (Kallio et al. 2010; Kallio et al. 2006b). With an increase of the common vole population, seen by higher number of trapped common voles (PAPER IV), and the decline of maternal antibodies in previously protected individuals, the number of susceptible common voles increase over the summer into fall, resulting in higher infection levels in fall compared to summer (PAPER IV). The susceptible reservoir density is one of the strongest drivers of hantavirus prevalence (Voutilainen et al. 2012). It is assumed that the density of the vole population in fall/winter can positively affect the spring density and that increased infection risk over winter can result in higher hantavirus prevalence in spring (Khalil et al. 2019; Voutilainen et al. 2016).

Table 3: Tula orthohantavirus prevalence in common vole (*M. arvalis*), Altai vole (*M. obscurus*) and field vole (*M. agrestis*) based on reverse transcription-PCR results.

Country	Species	Prevalence	Reference*
Austria	*M. arvalis*	13.3% (2/15)	(Schmidt et al. 2014)
China	*M. obsurus*	15.7% (31/198)	(Guo et al. 2019)
Croatia	*M. agrestis*	27.6% (8/29)	(Scharninghausen et al. 2002)
	M. arvalis	11.8% (12/102)	
Czech Republic	*M. arvalis*	n.d.	(Saxenhofer et al. 2019)
France	*M. arvalis*	23% (6/26)	(Schmidt et al. 2016)
Germany	*M. arvalis*	16.7% (3/18)	(Klempa et al. 2003a)
		9.1% (1/11)	(Mertens et al. 2011a)
		7.7% (1/13)	PAPER I
		13.8% (93/674)	PAPER IV
		n.d.	(Saxenhofer et al. 2019)
		15.8% (99/626)	(Schmidt et al. 2016)
	M. agrestis	2.4% (3/126)	PAPER I
		5.3 % (13/246)	(Schmidt et al. 2016)
Hungary	*M. arvalis*	37% (17/46)	(Jakab et al. 2008)
		6.2% (16/107)	(Kurucz et al. 2018)
Kazakhstan	*M. obscurus*	3.1% (4/128)	(Plyusnina et al. 2008a)
Netherland	*M. arvalis*	40.8% (20/49)	(Maas et al. 2017)
Slovenia	*M. arvalis*	33.3% (5/15)	(Korva et al. 2009)
		33.3% (5/15)	(Korva et al. 2013)
Slovakia	*M. arvalis*	5.6% (6/108)	(Sibold et al. 1999a)

n.d., not determined; *only studies with at least 10 sampled individuals were included

For TULV prevalence, strong geographic and local site discrepancies during the same season can be observed (PAPER I, Table 1 and PAPER IV, Schmidt et al., 2016). TULV is a reservoir-specific pathogen. This means that TULV prevalence can be affected by the dilution effect. Different habitats can result in varying biodiversity, with high biodiversity negatively affecting the TULV prevalence due to an increase of non-reservoir species abundance. In areas in the grassland habitat where species other than the common vole were captured, such as shrews, TULV was either not detected or its prevalence was very low (PAPER IV; Jeske et al., unpublished data). The dilution effect has been observed before with regard to European

hantaviruses in the bank vole-PUUV relationship (Khalil et al. 2016). In contrast to the effect observed in the areas within the grassland habitat, no dilution effect was detected between common voles captured at the forest edge, with higher biodiversity, and at the grassland habitat, with lower biodiversity. A reason for this might be found in the small number of trapped common voles at the forest edge (less than 10%) or in the study setting, where the distance between the corresponding trapping sites in grassland and forest edge habitat were not far enough from each other to detect an effect (PAPER IV).

The habitat represents another important factor to be studied in terms of pathogen prevalence. Habitats with abundant food resources for the reservoir can increase the reservoir abundance, which is positively correlated with the hantavirus prevalence (Augot et al. 2008). Vegetation providing coverage for common voles against predators can increase the vole dispersal and, with that, the chance to encounter the virus. In contrast, insufficient coverage, higher prevalence of predators or high vole abundance might be associated with higher stress levels. However, higher stress levels were surprisingly observed to be combined with higher level of immune defense against pathogens in voles (Jackson et al. 2011; Guivier et al. 2014). In addition, environmental conditions may affect the survival of the virus outside the host, influencing the probability of infection. Such factors can include UV light, soil, temperature and humidity (Monchatre-Leroy et al. 2017; Sauvage et al. 2002; Thoma et al. 2014).

Individual factors that influence hantavirus prevalence in voles include increase with age, for which weight is often used as a proxy (PAPER IV, Khalil et al. 2019; Khalil et al. 2016). Juvenile voles can be protected for a certain time by maternal antibodies if their mother was TULV-infected. It was reported for PUUV and the bank vole that this protection can last for up to two months (Kallio et al. 2010; Kallio et al. 2006b). No information is available yet on the duration of protection in common voles. Older voles become susceptible to TULV infection after a certain time, when they lose the maternal antibodies, and the probability of acquiring a TULV infection increases over time. No sex differences in susceptibility to TULV infection were observed (PAPER IV; Jeske et al., unpublished data). However, other studies have reported that males are more often infected with hantavirus than females, though this correlation is not always significant (Deter et al. 2008; Schmidt et al. 2016). The sex differentiation can probably be explained via the immunosuppressive effect of androgens and/or the relationships of steroid hormones with behaviors (aggressiveness, dispersal, foraging) that increase exposure to pathogens. As an example, tumor necrosis factor alpha (Tnf-α) expression is higher in female than in male bank voles and associated with lower PUUV loads (Guivier et al. 2014).

Another question is if only one species is the reservoir of TULV or if the virus can be hosted and spread by several reservoirs. TULV RNA has been detected in several Arvicolinae-species, especially *Microtus* voles, before (see Table 2 and Table 3, PAPER I). Several observations indicate that the common vole is the main reservoir of TULV in Central Europe. Even though other *Microtus* spp. are present in many areas where TULV was reported, detection of TULV RNA in other *Microtus* spp. is rare, compared to the high prevalence levels in common voles (PAPER IV, Jakab et al. 2008; Korva et al. 2009; Mertens et al. 2011a), and often combined in other *Microtus* spp. with higher prevalence in serological screening compared to common voles, indicating clearance of the virus instead of persistent infection (PAPER I, Korva et al. 2009; Korva et al. 2013; Schmidt et al. 2016). TULV sequences cluster, not species-specifically, but rather geographically (Schmidt et al. 2016) and are, at least for Central Europe, strongly associated with common vole lineages (Saxenhofer et al. 2019, Schmidt et al., 2016). The only exception is the Adler virus, a TULV-related hantavirus in the Major's pine vole that is genetically very different from TULV and clusters more basal in phylogenetic trees than any other TULV sequence (Tkachenko et al. 2015). Host spillover infection with TULV might occur due to sharing of the same habitat with common voles. Though it was proposed that field voles can act as a second reservoir species in the absence of common voles in some cases (PAPER I, Scharninghausen et al. 2002; Schmidt-Chanasit et al. 2010), this might be an effect of the fast radiation of the genus *Microtus*. It is likely that specific components of the immune system and receptors used by TULV might be very similar in closely related *Microtus* spp., therefore enhancing possible infection if contact with TULV occurs. Currently, no studies exist that compare TULV infection parameters such as virus shedding and duration of infection between common voles and other *Microtus* spp. In conclusion, the main reservoir of TULV is the common vole and, in regions where the common vole is absent, the sibling species Altai vole (*M. obscurus*) (Chen et al. 2019; Guo et al. 2019; Plyusnina et al. 2008a; Polat et al. 2018a).

4.1.3 Puumala orthohantavirus in Thuringia

PUUV was detected in Thuringia, Germany, (PAPER IV) in accordance with previous studies (Faber et al. 2019; Faber et al. 2013). This hantavirus is distributed in Europe in most areas where the bank vole is found, from France in the West (Castel et al. 2015) up to Russia in the East (Kariwa et al. 2009). The detection of the Central European PUUV lineage in Thuringia (PAPER IV) is in line with the detection of this lineage in other parts of Germany (Castel et al. 2019; Castel et al. 2015). The detected prevalence of 1.5% in Thuringia was very low (PAPER

IV), especially considering that PUUV prevalence in bank voles can reach high levels of up to 100% (Essbauer et al. 2007; Weber de Melo et al. 2015).

The PUUV prevalence in bank voles is affected by many factors such as the above-mentioned lineage, environmental factors, host abundance and season (see 4.1.2). The PUUV prevalence in Thuringia in 2017 increased from spring to fall (PAPER IV, Table 1), which is different from several studies in different years reporting highest prevalence in spring, followed by fall, and lowest prevalence in summer (Khalil et al. 2019; Voutilainen et al. 2016). The low prevalence detected in spring (PAPER IV) may indicate that the bank vole population density in fall of the previous year was low, as the density of the population in fall/winter for bank voles may affect the prevalence in spring (Khalil et al. 2019; Voutilainen et al. 2016). The abundance of bank voles should also not be a concern, as bank voles were the second most trapped rodent species, and 471 bank voles were sampled. They were present at most sites and seemed to be, based on trapping results, the dominant rodent species at some forest sites (PAPER IV, Table 1). A reason for the low prevalence might be the trapping location. Thuringia represents the eastern border of the PUUV range (Drewes et al. 2017a).

A study in Western Thuringia detected PUUV in bank vole populations, but the closer the sites were located to Central Thuringia, the more the prevalence decreased, until neither virus nor antibodies were detected (Faber et al. 2013). This might indicate that Central Thuringia is situated at the eastern border of the PUUV distribution range. Further studies are needed to show whether PUUV is still present in these areas and whether the distribution range is shifting. Furthermore, all positive-tested bank voles originated from four areas set at or close to the Hainich, a forest hill chain covering approximately 160 km², but PUUV sequences from there do not cluster with the geographically closest known PUUV sequences from Diedorf, Thuringia, but instead with sequences from bank voles that originated from the West and North of Germany (PAPER IV, Figure 2B). This might indicate that this area of the current federal state had been in-migrated by two different bank vole populations, one from the Northwest (resulting in the Hainich sequence type) and another from the South (resulting in the Diedorf sequence type).

4.1.4 Dobrava-Belgrade orthohantavirus and the striped field mouse

The striped field mouse population in Eurasia can carry DOBV genotype Kurkino. Though the reservoir is widely distributed from Central to Eastern Europe (see 1.2.1.), the virus was only previously reported for a handful of countries. These include Denmark, Germany, Hungary,

Slovakia, Slovenia and Russia (Kurucz et al. 2018; Nemirov et al. 2004; Plyusnin et al. 1999; Schlegel et al. 2009; Sibold et al. 1999b; Sibold et al. 2001). While no DOBV was detected in striped field mouse in Thuringia (PAPER IV), DOBV Kurkino was detected for the first time in Austria in the striped field mouse (PAPER V). The detection in Austria might be associated with the ongoing colonization of this reservoir host (Sackl et al. 2007; Spitzenberger 1997; Spitzenberger and Engelberger 2014). This finding is highlighted by the close similarity of the detected DOBV sequences to those from Sarmellek, Hungary (PAPER V, Figure 1B).

The DOBV prevalence in Austria (PAPER V) was lower than the reported prevalences from Croatia and Hungary of 3.8% up to 6.8%, respectively (Kurucz et al. 2018; Nemeth et al. 2011; Tadin et al. 2016). The reason for this might be the recent expansion of the striped field mouse. During the process of a range expansion, host-specific pathogens lag behind the expansion due low reservoir abundance as well as founder effects (Phillips et al. 2010; White et al. 2013).

While many countries such as Germany harbored the striped field mouse in the past, the expansion of the deciduous forest after the LGM expansion resulted in regression of the distribution range of this rodent. Later, human agricultural development resulted in occurrence of the preferred striped field mouse habitats and with these its re-colonization of Denmark, Germany and Italy, as well as more recently Czech Republic, Hungary, Slovenia and Austria (Spitzenberger and Engelberger 2014).

Surprisingly, DOBV was not detected in any of the 87 tested striped field mice from western Thuringia (PAPER IV). This hantavirus has been detected before in North and Eastern Germany in humans and striped field mice (Faber et al. 2019; Hofmann et al. 2014), and serological and molecular detection of DOBV in striped field mice in East Thuringia at the border to Saxony has occurred before (Rasche et al. 2015). The lack of reported human cases in Thuringia might indicate a very low prevalence of this pathogen. Otherwise, human cases would have been reported from Thuringia before. One reason might be that the distribution border of striped field mouse in Thuringia has been regressing (Spitzenberger and Engelberger 2014), possibly resulting in small, fragmented populations in which DOBV might have faded out.

4.2   Prevalence and host specificity of *Leptospira* spp. in Europe

4.2.1   *Leptospira* spp. prevalence in small mammals

Leptospira spp.-DNA was detected in Spain (PAPER III), Germany (PAPER IV) and Austria (PAPER V). Leptospires have been detected in several rodent species from different countries

in Europe before (Table 4). The prevalence varies with species, season, country and region as well as between sites within the same region (PAPER III, Table 1; PAPER IV, Table 1; PAPER V and Table 4). Some species, such as common vole and field vole, showed generally high prevalence of up to 54.5%, while most studies detected lower prevalence in comparison for mainly forest-dwelling rodents such as bank voles (Table 4). In contrast, one study in Croatia reported *Apodemus* spp. as more significant reservoirs than *Microtus* spp. (Tadin et al. 2016).

Table 4: *Leptospira* spp. prevalence, genomospecies and sequence types (ST) in different rodent species from Europe.

Family	Scientific name (common name)	Country	Detection rate (Prevalence)	Genomospecies and sequence type (ST)	Reference
Muridae	*Apodemus agrarius* (striped field mouse)	Austria	1/35 (2.9%)	*L. borgpetersenii* (n=1; ST 146, n=1)	PAPER V
		Croatia	12/53 (22.6%)	n.d.	(Tadin et al. 2016)
			10/29 (34.5%)	n.d.	(Stritof Majetic et al. 2014)
		Germany	27/296 (9.1%) 6/44 (13.6%)*	*L. kirschneri* (n=2; ST 127, n=2)	(Fischer et al. 2018a)
			3/7 (42.9%)	*L. kirschneri* (n=2)	(Obiegala et al. 2016)
			23/190 (12.1%)	*L. kirschneri* (n=19)	(Mayer-Scholl et al. 2014)
			19/86 (22.1%)	*L. kirschneri* (n=2)	PAPER IV
		Hungary	29/148 (19.6%)	n.d.	(Kurucz et al. 2018)
		Serbia	7/7 (100%)	n.d.	(Blagojevic et al. 2019)
	Apodemus flavicollis (yellow-necked mouse)	Austria	3/29 (10.3%)	*L. interrogans* (n=1; ST 297, n=1)	(PAPER V, Schmidt et al. 2014)
		Croatia	37/131 (28.2%)	n.d.	(Tadin et al. 2016)
			5/20 (25%)	n.d.	(Stritof Majetic et al. 2014)
		Germany	122/925 (13.2%)	*L. kirschneri* (n=19; ST 110, n=5), *L. borgpetersenii* (n=5; ST 197, n=2), *L. interrogans* (n=35; ST 24, n=15)	(Fischer et al. 2018a)

n.d., not determined; n, number of samples of the respective genomospecies or ST

Table 4 (continued)

Family	Scientific name (common name)	Country	Detection rate (Prevalence)	Genomospecies and sequence type (ST)	Reference
Muridae	*Apodemus flavicollis* (yellow-necked mouse)	Germany	27/296 (9.1%)*	[*L. kirschneri* (n=6; ST 110, n=4), *L. borgpetersenii* (n=4; ST 197, n=2), *L. interrogans* (n=2; St 24, n=1)]*	(Fischer et al. 2018a)
			15/247 (6.1%)	*L. kirschneri* (n=11) *L. borgpetersenii* (n=51) *L. interrogans* (n=36)	(Obiegala et al. 2016)
			2/4 (50%)	n.d.	(Obiegala et al. 2017a)
			55/345 (15.9%)	*L. kirschneri* (n=6) *L. borgpetersenii* (n=3)	PAPER IV
		Hungary	4/53 (7.5%)	n.d.	(Kurucz et al. 2018)
		Serbia	12/35 (34.3%)	n.d.	(Blagojevic et al. 2019)
		Sweden	1/5 (20)	n.d.	(Backhans et al. 2013)
	Apodemus sylvaticus (wood mouse)	Austria	2/26 (7.7%)	n.d.	(Schmidt et al. 2014), PAPER V**
		Croatia	3/9 (33.3%)	n.d.	(Stritof Majetic et al. 2014)
		Germany	9/105 (8.6%) 1/16 (6.3%)*	*L. interrogans* (n=4; ST 24, n=3)	(Fischer et al. 2018a)
			27/154 (17.5%)	*L. kirschneri* (n=6) *L. interrogans* (n=13)	(Mayer-Scholl et al. 2014)
			8/60 (13.3%)	*L. kirschneri* (n=2)	PAPER IV
		Hungary	1/29 (3.4%)	n.d.	(Kurucz et al. 2018)
		Italy	2/13 (15.4%)	n.d.	(Vitale et al. 2018)
		Spain	20/266 (11.3%)	*L. kirschneri* (n=5) *L. borgpetersenii* (n=12) *L. interrogans* (n=13)	(Millan et al. 2018)
Cricetidae	*Microtus agrestis* (field vole)	Germany	62/209 (29.7%) 1 58/178 (32.1%)*	*L. kirschneri* (n=43; ST 110, n=32) *L. kirschneri* (n=41)*	(Fischer et al. 2018a)
			64/517 (12.4%)	*L. kirschneri* (n=38) *L. borgpetersenii* (n=6) *L. interrogans* (n=1)	(Mayer-Scholl et al. 2014)
			6/11 (54.5%)	*L. kirschneri* (n=1)	PAPER IV
	Microtus arvalis (common vole)	Austria	1/15 (6.7%)	*L. kirschneri* (n=1; ST 110, n=1)	(Schmidt et al. 2014), PAPER V**
		Croatia	1/4 (25%)	n.d.	(Tadin et al. 2016)
			1/4 (25%)	n.d.	(Tadin et al. 2016)
			1/1 (100%)	n.d.	(Stritof Majetic et al. 2014)
		Germany	113/377 (30.0%)	*L. kirschneri* (n=84; ST 110, n=25)	(Fischer et al. 2018a)
			28/121 (23.1%)*	*L. kirschneri* (n=21)*	

n.d., not determined; n, number of samples of the respective genomospecies or ST; *previous results from Gotha, Thuringia, Germany; ** *Leptospira* spp. screening in Schmidt et al., 2014 and typing in PAPER V

Table 4 (continued)

Family	Scientific name (common name)	Country	Detection rate (Prevalence)	Genomospecies and sequence type (ST)	Reference
Cricetidae	*Microtus arvalis* (common vole)	Germany	24/174 (13.8%)	*L. kirschneri* (n=19)	(Mayer-Scholl et al. 2014)
			3/7 (42.9%)	*L. kirschneri* (n=2)	(Obiegala et al. 2016)
			205/676 (30.3%)	*L. kirschneri* (n=92)	PAPER IV
		Hungary	27/107 (25.2%)	n.d.	(Kurucz et al. 2018)
		Serbia	5/21 (23.8%)	n.d.	(Blagojevic et al. 2019)
		Spain	46/580 (7.9%)	*L. kirschneri* (n=24) *L. borgpetersenii* (n=2)	PAPER III
	Microtus lusitanicus (Lusitanian pine vole)	Spain	2/6 (33.3%)	n.d.	PAPER III
	Microtus subterraneus (European pine vole)	Serbia	1/7 (14.3%)	n.d.	(Blagojevic et al. 2019)
	Clethrionomys glareolus (bank vole)	Austria	3/39 (7.7%)	*L. kirschneri* (n=1; ST 110, n=1)	(Schmidt et al. 2014), PAPER V**
		Croatia	1/43 (2.3%)	n.d.	(Tadin et al. 2016)
			1/1 (100%)	n.d.	(Stritof Majetic et al. 2014)
		Germany	13/1578 (7.8%)	*L. kirschneri* (n=11; ST 110, n=6), *L. borgpetersenii* (n=7; ST 146, n=3, ST 197; n=1)	(Fischer et al. 2018a)
			28/486 (5.8%)*	*L. interrogans* (n=40, ST 24; n=27) [*L. kirschneri* (n=6, ST 110; n=5), *L. borgpetersenii* (n=1), *L. interrogans* (n=2, ST 24; n=1)]*	
			66/1016 (6.5%)	*L. kirschneri* (n=38) *L. interrogans* (n=18)	(Mayer-Scholl et al. 2014)
			39/737 (5.3%)	*L. kirschneri* (n=34) *L. interrogans* (n=2)	(Obiegala et al. 2016)
			27/660 (4.1%)	n.d.	(Obiegala et al. 2017a)
			54/474 (11.4%)	*L. kirschneri* (n=4) *L. borgpetersenii* (n=5)	PAPER IV
		Hungary	1/1 (100%)	n.d.	(Kurucz et al. 2018)
		Serbia	9/17 (52.9%)	n.d.	(Blagojevic et al. 2019)

n.d., not determined; n, number of samples of the respective genomospecies or ST; *previous results from Gotha, Thuringia, Germany; ** *Leptospira* spp. screening in Schmidt et al., 2014 and typing in PAPER V

4.2.2 Rodent-specificity of *Leptospira* spp. sequence type

Studies in the past often screened only for the presence of pathogenic *Leptospira* spp. by usage of *lipl32*-PCR. Recent studies additionally type the *Leptospira* spp. using *secY*-PCR as well as MLST. This resulted in the detection of the genomospecies *L. kirschneri*, *L. borgpetersenii* and *L. interrogans* as well as further ST in European screening studies that are associated with different rodent species (Fischer et al. 2018a; Mayer-Scholl et al. 2014; Millan et al. 2018; Obiegala et al. 2016; Stritof Majetic et al. 2014). Field-inhabiting rodents, especially the common vole, but also the field vole and striped field mouse, are usually reported to be infected with *L. kirschneri*, while forest-dwelling rodents harbored a mixture of up to three genomospecies (Table 4). Common voles were almost exclusively reported with *L. kirschneri* ST 110 infection (PAPER III, PAPER IV, Table 4), except for one area in Baden-Wurttemberg, Germany, where ST 230 was detected (Fischer et al. 2018a), indicating that this species is an important reservoir of this sequence type. In Spain, 92% of leptospires from common voles were typed as genomospecies *L. kirschneri*. Contary to the trend toward exclusive detection of this genomospecies, two common voles harbored *L. borgpetersenii* instead (PAPER III). Infection with *L. borgpetersenii* is usually reported from forest-dwelling rodents, such as bank voles and yellow-necked mice, but was also detected from rodents that can dwell in fields such as striped field mouse and field vole (Table 4). A spillover infection of the two common voles through contact with wood mice, which were also trapped in the same areas, is likely (PAPER III), as this rodent species has been detected with *L. borgpetersenii* before in Spain (Millan et al. 2018). In the recent study in Thuringia, *L. interrogans* was not detected in forest-dwelling rodents (PAPER IV), although it has been reported before (Fischer et al. 2018a; Mayer-Scholl et al. 2014; Obiegala et al. 2016). In the recent study, *L. interrogans* with a new ST was detected in a yellow-necked mouse from Austria (PAPER V). Only *L. kirschneri* ST 110 was generally reported in Spain, Germany and Austria, especially in the common vole, indicating a broad Europe-wide distribution (PAPER III, PAPER IV, and PAPER V).

Various studies indicate a discrepancy between the results of *lipl32*-PCR screening and typing by *secY*-PCR (PAPER III and PAPER IV; Fischer et al. 2018a; Mayer-Scholl et al. 2014; Millan et al. 2018; Obiegala et al. 2016; Stritof Majetic et al. 2014). In fact, only 33.1-54.2% of the initially positive-tested samples were successfully typed (PAPER III and PAPER IV). A potential explanation might be that the *lipl32*-PCR has a lower specificity, since the *lipl32* sequence can also occur in non-*Leptospira* bacteria (Gamage et al. 2020).

4.2.3 Factors that affect *Leptospira* spp. prevalence

Individual factors that affect prevalence levels include weight, as a proxy for age (PAPER III; Costa et al. 2014; Fischer et al. 2018a; Krøjgaard et al. 2009). Once infected, rodent reservoirs are believed to be lifelong carriers (Adler and de la Pena Moctezuma 2010), hence resulting in an increase of infection rate over time. Sex of the rodent was not identified as a factor for *Leptospira* spp. infection (PAPER III; PAPER IV; Benacer et al. 2016; Kurucz et al. 2018).

Leptospira spp. prevalence was reported to show annual and seasonal variation (PAPER III; PAPER IV; Fischer et al. 2018a; Kurucz et al. 2018). While seasonality has been described before for species-specific pathogens that cause lifelong infection of the reservoir, such as hantaviruses (see 4.2.2), the seasonality for *Leptospira* spp. infection is different. This is true even when both pathogens were screened in parallel in the same population (PAPER IV), indicating that environmental or currently unknown factors are of great importance for understanding the variation of *Leptospira* spp. prevalence. Studies on seasonality of *Leptospira* spp. in rodents from Europe are limited to Germany and Hungary, but they indicate increase from spring throughout the summer to fall (PAPER IV, Fischer et al. 2018a; Kurucz et al. 2018). High reservoir abundance combined with high pathogen prevalence in the previous fall/winter were reported to result in high prevalence in spring for other pathogens that cause lifelong infection of the reservoir (Voutilainen et al. 2016). In contrast to that, the spring prevalence for *Leptospira* spp. was very low (PAPER IV, Fischer et al. 2018a). A reason might be a decreased survival rate of *Leptospira* spp.-infected reservoirs compared to uninfected animals in winter, resulting in a low number of infected individuals in spring. Additionally, winter conditions might negatively affect the survival of leptospires in the environment, as they are sensitive to cold temperature (Andre-Fontaine et al. 2015), resulting in fewer infections. Furthermore, *Leptospira* spp. infection in some wild rodents might not result in lifelong infection/shedding, which has been reported before for rats (Monahan et al. 2008), but rather in clearance or in infection levels decreased to below the PCR threshold. The pathogen load during chronic infection might furthermore vary depending on *Leptospira* spp. ST and reservoir and is currently unknown for various rodent reservoirs. Even shedding of leptospires by *Rattus norvegicus* can differ greatly between *L. interrogans* isolates, ranging from 40 days to 2 to 4 months or up to 220 days (Athanazio et al. 2008; Nally et al. 2005; Thiermann 1981).

The increase of prevalence from spring to fall might be reservoir abundance-related, as the observed increase in the common vole population up to fall was combined with higher

Leptospira spp. prevalence (PAPER IV). A link between density, dominance index and/or population cycle was also seen for *Leptospira* spp. in common voles in Spain (PAPER III). The dominance index might play an important role, because some *Leptospira* spp. and their ST, i.e. *L. kirschneri* ST 110, seem to be reservoir species-specific (see 4.2.2.). Therefore, *Leptospira* spp. in some hosts may be affected by the small mammal biodiversity, which can be influenced by the habitat.

Besides the influence of the reservoir population dynamics, environmental and weather factors in particular are important for the *Leptospira* spp. prevalence. From spring to summer, the temperature increases in Europe. This is important because leptospires are temperature-sensitive bacteria that need to survive and retain infectivity outside their host until they can infect a new host. The optimal temperature is around 30°C, with decreasing survival with lower temperature (Andre-Fontaine et al. 2015). In warm, tropical countries, where *Leptospira* spp. is endemic and temperatures are not as fluctuating as in Europe, a rainy season can positively, while dry season can negatively, affect the *Leptospira* spp. prevalence in rodents (Benacer et al. 2016; Cortez et al. 2018; Ivanova et al. 2012; Mason et al. 2016). Warm and rainy seasons result in the highest prevalence in rodents in New Caledonia (Perez et al. 2011), dogs in the USA (Ward 2002) and cattle in Australia (Carroll and Campbell 1987). In Spain, the best minimum adequate model to calculate *Leptospira* spp. prevalence in common voles include common vole weight, a temperature of 27°C degree and a high level of accumulated rainfall in the last 90 days before rodent trapping (PAPER III). Rainfall, as well as irrigation ditches, rivers, flooding and bodies of standing, can increase the ground moisture. This in turn can enhance the wild grass seed production, increasing the reservoir densities and therefore indirectly the *Leptospira* spp. prevalence (Diaz 2015) as well as the *Leptospira* spp. survival outside the host, as humidity is essential for leptospires (Cortez et al. 2018; Vitale et al. 2018). Other factors that affect *Leptospira* spp. survival outside the host might include soil composition (salt concentration and pH) (Thibeaux et al. 2018a). This might be an important factor in the observed differences between trapping locations (PAPER III and PAPER IV, Fischer et al. 2018a). In addition, the vegetation can affect the population composition and density and the reservoir behavior by providing coverage from predators. Droughts might negatively affect *Leptospira* spp. prevalence by two mechanisms: first, by decreasing ground moisture, negatively affecting leptospires' survival outside the host, and secondly, by negatively affecting food resources such as grass, thus decreasing the rodent population density.

4.3　Detection of other, vector-borne pathogens

In Austria, striped field mice were screened for, besides *Leptospira* spp. and DOBV, additional pathogens that are transmitted by blood-sucking arthropods like fleas and ticks. These include *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Bartonella* spp., *Borrelia* spp. and *Neoehrlichia mikurensis*. Several of these pathogens were previously known from ticks in Austria (Glatz et al. 2014; Schötta et al. 2017), but not from striped field mice. In 12.5% of tested striped field mice from Austria, *Bartonella taylorii* and a novel *Bartonella* species were detected, and in 1.3% *Borrelia miyamotoi* and in 6.3% *Neoehrlichia mikurensis* were detected.

Table 5:　Molecular detection of *Bartonella* spp. in the striped field mouse (*Apodemus agrarius*)

Country	*Bartonella* species	Prevalence	Reference
Austria	n.d.	12.5%	PAPER IV
China	n.d.	46.9%	(Liu et al. 2010)
	B. fuyuanensis	11.1%	(Qin et al. 2019)
Croatia	New *A. agrarius*-associated cluster, and *B. rochalimae*-associated	13.2%	(Tadin et al. 2016)
Korea	n.d.	6.3%	(Chae et al. 2008)
	B. grahamii, B. taylorii, B. tribocorum, B. phoceensis, B. henselae,	31.5/62%*	(Ko et al. 2016)
Poland	*B. grahamii, B. taylorii,* and *B. birtlesii*, *B. elizabethae*-like	38.3%	(Hildebrand et al. 2013)
Russia	n.d.	93%	(Mediannikov et al. 2005)
Slovakia	*B. grahamii, B. taylorii, B. birtlesii. B. clarridgeiae/B. rochalimae* clade, *B. elizabethae/B. tribocorum* clade	9%	(Kraljik et al. 2016)
Slovenia	*B. grahamii*	26.6%	(Knap et al. 2007)

n.d., not determined, * depending on PCR used

Bartonella spp. prevalence in Austrian striped field mice (PAPER IV) is very low compared to the results of other studies that screened striped field mice, but it is comparable to the prevalence detected in rodents from another study in Austria (Schmidt et al. 2014). There, *Bartonella* spp. was detected in three rodent species: yellow-necked mouse (10.7% with *B. taylorii*), wood mice

(15.4% *B. taylorii* and 3.8% *B. birtlesii*) and common voles (13.3% *B. taylorii*) (Schmidt et al. 2014). Most of the reported *Bartonella* spp. from striped field mouse, except *B. henselae* which is a known zoonotic species (Mada et al. 2020), as well as many of the other reported *Bartonella* spp. from other rodents in Central Europe have unknown zoonotic potential (Obiegala et al. 2019).

Borreliosis disease (also called Lyme disease) is caused by *Borrelia* spp., especially *B. afzelii* in Europe, which is carried by *Ixodes ricinus* ticks and rodents (Richter et al. 2004). *Borrelia* spp. were detected in striped field mouse with prevalence ranging from 1.9% to 42.9% (Table 6), therefore the observed prevalence in Austria is very low (PAPER V). The detection of *Borrelia* spp. is in line with the fact that borreliosis is endemic in Austria (Khanakah et al. 2006). *Borrelia* spp. were previously detected in several rodent species in Austria with prevalence ranging from 3.8% to 45.5% depending on species and study, but it had not been detected in striped field mouse before (Khanakah et al. 2006; Schmidt et al. 2014). *Borrelia miyamotoi*, detected in rodents, including *Apodemus* spp., can cause relapsing fever in humans (Cutler et al. 2019). This *Borrelia* spp. was detected from one striped field mouse in Austria as well as from an Austrian patient (PAPER V, Tobudic et al. 2020).

Table 6: Prevalence of *Borrelia* spp. in striped field mouse (*Apodemus agrarius*).

Country	*Borrelia* species	Prevalence	Reference
Austria	*B. miyamotoi*	1.3%	PAPER IV
China	*B. valaisiana*	n.d.	(Masuzawa et al. 2001)
	B. garnii	23.3%	(Zhang et al. 2010)
Croatia	*B. afzelii*/ *B. miyamotoi*	1.9%/13.2%	(Tadin et al. 2016)
Germany	n.d.	42.9%**	(Obiegala et al. 2017b)
Korea	*B. burgdoferi* sensu lato	8.3%*	(Park et al. 1993)
Poland	*B. afzelii*	7.6%	(Gryczyńska et al. 2018)
Taiwan	*B. valaisiana*	n.d.	(Masuzawa et al. 2000)

n.d., not determined; * isolation; ** only seven striped field mice tested

Neoehrlichia mikurensis has been detected before in striped field mouse in Korea with a prevalence of 28.6% (Jha et al. 2018), as well as in Hungary (Szekeres et al. 2015). This bacterium was reported for the first time in a rodent from Austria (PAPER V) but had been detected before in ticks (Glatz et al. 2014). While the infection is usually asymptomatic in

humans, inflammatory infection was reported for immunocompromised patients (Portillo et al. 2018).

In the recent study, *Babesia microti*, *Anaplasma* spp. and *Ehrlichia* spp. were not detected in striped field mice from Austria (PAPER V), possible due to the low prevalence reported for this rodent species in general.

Babesia microti was previously detected in striped field mice with very low prevalence of 0.52% in Korea (Hong et al. 2014) as well as 5.7% in Croatia (Tadin et al. 2016). For *A. phagocytophilum*, the prevalence in striped field mouse in Korea was 5.6% (Chae et al. 2008). Striped field mice from Korea were tested by a PCR detecting both *Anaplasma* spp. and *Ehrlichia* spp., resulting in 72.3% positive-tested striped field mice. By a separate PCR, 23.6% of striped field mice were tested positive for *A. phagocytophilum* (Kim et al. 2006). In Croatia, 1.9% of tested striped field mice were positive for *Ehrlichia* spp. but not for *Anaplasma* spp. (Tadin et al. 2016). There seems to be no major difference between the separate striped field mouse populations in Europe and Asia (Latinne et al., 2020) but rather strong differences between studies for vector-borne pathogens in general, which might be due to seasonal or other factors.

4.4 Coinfections of *Leptospira* spp., hantavirus and other pathogens

Rodents can harbor more than 60 different pathogens (Meerburg et al. 2009). In the common vole as an example, 3 fungi (Table S1), 9 viruses (Table S2), 25 bacteria (Table S3) and 24 parasites (Table S4) have been detected. Some of these such as the novel common vole-associated hepevirus have unknown pathogenic potential to humans (Ryll et al. 2019). The common vole can act as an intermediate host for some pathogens, such as parasites like *Toxoplasma gondii* (Führer et al. 2010).

To detect possible coinfections, rodents were screened for *Leptospira* spp. and hantavirus infection in Spain (PAPER III), Germany (PAPER IV) and Austria (PAPER V). In Spain, no coinfections could be detected, due to apparent absence of TULV on the Iberian Peninsula (PAPER III). Absence of coinfection of *Leptospira* spp. and hantaviruses in Austria might be linked to the low prevalence of the detected pathogens in striped field mice. For Austria, coinfection of *Neoehrlichia mikurensis* and *Bartonella* spp. was observed instead (PAPER V). Multiple co- and triple infections of *Borrelia azfelii* combined with *Leptospira* spp., TULV, Rickettsia and *Bartonella* spp. were also reported from Austrian rodents (Schmidt et al. 2014).

In Thuringia, Germany, coinfection of *Leptospira* spp. and hantavirus was observed in 6.6% of common voles as well as 0.5% of tested bank voles (PAPER IV). Though cowpox virus infection was detected in five bank voles (0.1%) in Thuringia, Germany in 2017 (Jeske et al. 2019b; Jeske et al., unpublished data), no coinfections with *Leptospira* spp. (11.4%) or PUUV (1.5%) were detected (PAPER IV; Jeske et al., unpublished data), probably due to the low prevalence of cowpox virus and PUUV. Sometimes, coinfection can be more likely than infection with a single pathogen or more likely than statistics would suggest, as observed for bank voles and rats (Ayral et al. 2015; Obiegala et al. 2017a). Other factors include increase with age, which was previously reported to be associated with separate infections by both pathogens (PAPER IV; Costa et al. 2014). Prevalence of coinfection increased from spring to fall in association with increased *Leptospira* spp. prevalence, because the latter is an important driving factor for coinfection levels (PAPER IV). This is in line with frequency of coinfections in bank voles, where 50% of coinfected individuals were trapped at an area with high *Leptospira* spp. prevalence (Obiegala et al. 2017a).

In a study with 15 screened pathogens, half of the rodents were positive for at least one pathogen, 11% for a double infection and 3% for a triple infection. From these, *Leptospira* spp. was the most common detected pathogen, followed by hantaviruses and *Bartonella* spp. (Tadin et al. 2016). In another study with rats and 17 screened pathogens, 87.5% of screened rats were shown to be infected with at least two pathogens (Desvars-Larrive et al. 2017). Metagenomic studies of intestine samples from 20 Norway rats resulted in the detection of multiple viral coinfections (Sachsenröder et al. 2014). These findings indicate that coinfection is very common in nature and should be further analyzed. *Leptospira* spp. and hantaviruses are two globally distributed pathogens with high prevalences in their respective hosts (Vijayachari et al. 2008), making coinfection with both pathogens in their respective reservoir likely. *Leptospira* spp. coinfections have been reported before in rats (*R. norvegicus*) with SEOV, in yellow-necked mouse and striped field mouse with DOBV, in striped field mouse with HTNV, in common voles and one spillover-infected bank vole with TULV, and in bank voles with PUUV (see Table 7).

Though rare, studies exist that link new infection risk with already existing infections in the reservoir. Field voles were screened for four pathogens (cowpox virus, *Babesia microti*, *Bartonella* spp. and *Anaplasma phagocytophilum*) and several interactions between the pathogens, from positive to negative, were detected that were as strong as seasonal effects. Cowpox virus infection was reported to increase due to immunomodulatory effects that increase

Table 7: *Leptospira* spp.-hantavirus coinfections in rodents.

Species	Country	*Leptospira* genomospecies	Hantavirus	Additional pathogen	Prevalence of double (triple or quadruple) infection	Reference
Apodemus agrarius	Croatia	n.d.	DOBV	*Bartonella* spp., *Francisella tularensis*	n.a. (1.9%)	(Tadin et al. 2016)
	Hungary	n.d.	DOBV		n.a.	(Kurucz et al. 2018)
	Korea	n.d.	HTNV		0.7%	(Park et al. 2016)
Apodemus flavicollis	Croatia	Serogroup Australis/ Grippoty-phosa*	DOBV	*Babesia* spp.	53.6% (10.7%)	(Tadin et al. 2012)
Apodemus flavicollis	Croatia	n.d.	DOBV	*Bartonella* spp. *Borrelia azfelii*	7.6% (0.8%)	(Tadin et al. 2016)
Clethrionomys glareolus	Croatia	*L. interrogans* serovar lora	PUUV		1.8%	(Cvetko et al. 2006)
		n.d.*	PUUV		6.3%	(Tadin et al. 2012)
	Germany	n.d.	PUUV		n.a.	(Obiegala et al. 2017a)
	Hungary	n.d.	TULV		100% [1/1]	(Kurucz et al. 2018)
Microtus arvalis	Croatia	n.d.	TULV		25% [1/4]	(Tadin et al. 2016)
	Hungary	n.d.	TULV		3.7%	(Kurucz et al. 2018)
Rattus norvegicus	Brazil	*L. interrogans*	SEOV	-	13.8%	(Costa et al. 2014)
	France	*L. interrogans*	SEOV	Hepatitis E virus	n.a.	(Ayral et al. 2015)

n.a., not available; n.d., not determined; * detection by serology; [number of positive tested rodents/number of tested rodents], when less than less than ten rodents were screened; DOBV, Dobrava-Belgrade orthohantavirus; HTNV, Hantaan orthohantavirus; PUUV, Puumala orthohantavirus; SEOV, Seoul orthohantavirus; TULV, Tula orthohantavirus

the susceptibility to other infections, while other pathogens were reported to reduce susceptibility due to resource depletion (Telfer et al. 2010). All of these pathogens were already reported for common voles (Tables S1 - S4), sometimes with very high prevalences such as that for *Babesia microti* (Sinski et al. 2006; Tolkacz et al. 2017). Also detected in common voles,

helminths such as *Trichuris arvicolinae* (Table S4) are known to increase susceptibility to bacterial (Pathak et al. 2012), parasitic (malaria) (Knowles 2011) and viral infections (Graham 2008; Kamal and El Sayed Khalifa 2006) through immunomodulatory processes. Bank voles infected with the helminth *Heligmosum mixtum* exhibit higher PUUV loads than helminth-free bank voles (Guivier et al. 2014). Over one quarter of common voles were infected in a study with another *Heligmosum* spp., *Heligmosomum costellatum* (Janova et al. 2010), making it likely that similar effects might also be observed for TULV.

Whether *Leptospira* spp. can promote hantavirus infection through immunomodulation is unknown. They are known to evade complement activation through several means such as inactivation of complement proteins in the surroundings (Fraga et al. 2016). While *Leptospira* spp. proliferate in the organism during the first days, the infection is soon controlled and leptospires are cleared from the blood. They are afterwards only detected at the proximal renal tubules (Ratet et al. 2014), making promotion of hantavirus infections unlikely.

5 Outlook

The investigations of voles from Germany and Lithuania resulted in the discovery of two novel virus strains, i.e. TRAV and RUSV, which both belong taxonomically to the putative species "Tatenale orthohantavirus". Future investigations, such as in Northern Poland, should evaluate the geographical range of this virus and its strains, the host specificity and adaptation of this virus species and its strains and the evolutionary history of its distribution in Europe. The production of a yeast-expressed RUSV N protein will enable serological studies in humans to be performed to evaluate the zoonotic potential of this virus. However, this ELISA-based approach must be accompanied by a neutralization assay based on a virus isolate or heterologous recombinant virus-like particles with the GPC of TATV. Alternatively, a highly sensitive real-time RT-PCR assay should be developed to detect potential human infections during the acute phase. Usage of isolated virus and/or of virus-like particles with TATV glycoproteins would allow cell-receptor binding experiments. The results could help to evaluate the risk to humans.

The detection of PUUV in a region in western Thuringia raised questions about the origin of this strain and the strain found earlier within a 50-km distance. The current phylogenetic data suggest a different origin of these two PUUV lineages, but further proof in the form of additional and full-genome PUUV sequences is needed. Future investigations will need to map the current distribution range of PUUV at its eastern border in Thuringia and to elucidate the evolutionary processes active during host-mediated virus "migration". These studies should be accompanied by bank vole population studies using mitochondrial cytochrome *b* sequences. In the case that these are not sufficient, genomic microsatellite markers might help to enable a more detailed genetic differentiation of bank vole populations in Thuringia. Similarly, the lack of evidence of DOBV in the western part of Thuringia, despite its occurrence in eastern Thuringia, raised the question of the distribution range of this virus. Although striped field mice were collected at the sites in western Thuringia, DOBV was not detected at all. Here, more systematic transect-based monitoring studies are needed during a longer time period to map the border of the DOBV distribution range. Important factors to analyze might furthermore contain striped field mouse abundance, which might have been too low to maintain DOBV, or the presence of fragmented, small populations in which DOBV has faded out. The first detection of DOBV in Austria and the ongoing range expansion of the striped field mouse should be systematically followed to understand migration processes and the influence of pathogens on these processes. Consequently, the previously observed "migration" of pathogens compared to

non-infected reservoir individuals raises questions regarding the pathogenicity of PUUV and DOBV in the respective reservoir hosts. Here, further studies in wild-trapped rodents, but also experimental infection studies in these rodent species, are needed to find out potential consequences of the infections on the fitness of the reservoir.

The similarities of the clinical outcome of hantavirus and *Leptospira* infections obviously required further efforts to study both pathogens. *Leptospira* spp.-DNA screening resulted in the detection of this pathogen in Spain, Germany and Austria. The finding of *L. kirschneri* in common voles in Spain and Thuringia confirmed previous studies, but it also raises novel questions on the host specificity of *Leptospira* genomospecies. The discrepancy between *Leptospira* spp. screening and typing results needs further investigation, which could involve the development and validation of novel screening PCRs based on e.g. 16S rRNA and/or typing approaches such as those based on next generation sequencing. More information on genomospecies would enable determination of whether different *Leptospira* spp. ST are associated with different rodent species as well as determination of the distribution of rare and new genotypes.

Interestingly, for both pathogens, even similarities in their ecology have been identified – both pathogens seem to cause persistent infections resulting in increased prevalences in older individuals. However, the frequency of infections differ in seasonality. Ecological drivers of the prevalence of these pathogens, such as biodiversity, landscape features and land use, urgently needed to be studied in more detail, as they are strongly influenced by human activity in different parts of the world. For *Leptospira* spp., particularly the effect of water (rainfall, distance to water and ground humidity), in addition to other environmental and population-based factors, should be studied in more detail.

Moreover, the frequency of double infection of reservoirs, such as *Leptospira* spp.-hantavirus coinfection, needs additional attention. As a single pathogen infection may influence the likelihood of infection with other pathogens, future studies need to evaluate not only factors that influence the prevalence of a single pathogen but also the interaction between pathogens. These studies should take the whole pattern of the infectious agents, including the intestine microbiome, within a reservoir into account, and its effects on the fitness of the reservoir.

References

Achazi, K., Ruzek, D., Donoso-Mantke, O., Schlegel, M., Ali, H. S., Wenk, M., Schmidt-Chanasit, J., Ohlmeyer, L., Ruhe, F., Vor, T., Kiffner, C., Kallies, R., Ulrich, R. G. and Niedrig, M. (2011). Rodents as sentinels for the prevalence of tick-borne encephalitis virus. Vector Borne Zoonotic Dis *11 (6)*, 641-647, doi: 10.1089/vbz.2010.0236.

Adler, B. (2015). Vaccines against leptospirosis. Curr Top Microbiol Immunol *387*, 251-272, doi: 10.1007/978-3-662-45059-8_10.

Adler, B. and de la Pena Moctezuma, A. (2010). Leptospira and leptospirosis. Vet Microbiol *140 (3-4)*, 287-296, doi: 10.1016/j.vetmic.2009.03.012.

Ali, H. S., Drewes, S., Sadowska, E. T., Mikowska, M., Groschup, M. H., Heckel, G., Koteja, P. and Ulrich, R. G. (2014). First molecular evidence for Puumala hantavirus in Poland. Viruses *6 (1)*, 340-353, doi: 10.3390/v6010340.

Amori, G., Hutterer, R., Kryštufek, B., Yigit, N., Mitsain, G. and Palomo, L.J. (2016). *Apodemus flavicollis* (errata version published in 2017). URL: https://www.iucnred list.org/species/1892/115058023 [as of 24.05.2020].

Amori, G., Hutterer, R., Yigit, N., Mitsain, G., Kryštufek, B., Vohralík, V., Zima, J. and Zagorodnyuk, I. (2016). *Microtus subterraneus* (errata version published in 2017). URL: https://www.iucnredlist.org/species/13489/115115111 [as of 24.05.2020].

Andersen-Ranberg, E. U., Pipper, C. and Jensen, P. M. (2016). GLOBAL PATTERNS OF LEPTOSPIRA PREVALENCE IN VERTEBRATE RESERVOIR HOSTS. J Wildl Dis *52 (3)*, 468-477, doi: 10.7589/2014-10-245.

Andre-Fontaine, G., Aviat, F. and Thorin, C. (2015). Waterborne Leptospirosis: Survival and Preservation of the Virulence of Pathogenic *Leptospira* spp. in Fresh Water. Curr Microbiol *71 (1)*, 136-142, doi: 10.1007/s00284-015-0836-4.

Antoniadis, A., Stylianakis, A., Papa, A., Alexiou-Daniel, S., Lampropoulos, A., Nichol, S. T., Peters, C. J. and Spiropoulou, C. F. (1996). Direct genetic detection of Dobrava virus in Greek and Albanian patients with hemorrhagic fever with renal syndrome. J Infect Dis *174 (2)*, 407-410, doi: 10.1093/infdis/174.2.407.

Athanazio, D. A., Silva, E. F., Santos, C. S., Rocha, G. M., Vannier-Santos, M. A., McBride, A. J., Ko, A. I. and Reis, M. G. (2008). Rattus norvegicus as a model for persistent renal colonization by pathogenic *Leptospira interrogans*. Acta Trop *105 (2)*, 176-180, doi: 10.1016/j.actatropica.2007.10.012.

Augot, D., Sauvage, F., Boue, F., Bouloy, M., Artois, M., Demerson, J. M., Combes, B., Coudrier, D., Zeller, H., Cliquet, F. and Pontier, D. (2008). Spatial and temporal patterning of bank vole demography and the epidemiology of the Puumala hantavirus in northeastern France. Epidemiol Infect *136 (12)*, 1638-1643, doi: 10.1017/s0950268808000423.

Aulagnier, S. (2016). *Microtus lusitanicus*. URL: https://www.iucnredlist.org/species/13494/ 513980 [as of 24.05.2020].

Avsic-Zupanc, T., Nemirov, K., Petrovec, M., Trilar, T., Poljak, M., Vaheri, A. and Plyusnin, A. (2000). Genetic analysis of wild-type Dobrava hantavirus in Slovenia: co-existence of two distinct genetic lineages within the same natural focus. J Gen Virol *81 (Pt 7)*, 1747-1755, doi: 10.1099/0022-1317-81-7-1747.

Avsic-Zupanc, T., Toney, A., Anderson, K., Chu, Y.-K. and Schmaljohn, C. (1995). Genetic and antigenic properties of Dobrava virus: a unique member of the *Hantavirus* genus, family *Bunyaviridae*. J Gen Virol *76 (11)*, 2801-2808.

Avsic-Zupanc, T., Xiao, S. Y., Stojanovic, R., Gligic, A., van der Groen, G. and LeDuc, J. W. (1992). Characterization of Dobrava virus: a Hantavirus from Slovenia, Yugoslavia. J Med Virol *38 (2)*, 132-137, doi: 10.1002/jmv.1890380211.

Ayral, F., Artois, J., Zilber, A. L., Widen, F., Pounder, K. C., Aubert, D., Bicout, D. J. and Artois, M. (2015). The relationship between socioeconomic indices and potentially zoonotic pathogens carried by wild Norway rats: a survey in Rhone, France (2010-2012). Epidemiol Infect *143 (3)*, 586-599, doi: 10.1017/s0950268814001137.

Backhans, A., Jacobson, M., Hansson, I., Lebbad, M., Lambertz, S. T., Gammelgård, E., Saager, M., Akande, O. and Fellström, C. (2013). Occurrence of pathogens in wild rodents caught on Swedish pig and chicken farms. Epidemiol Infect *141 (9)*, 1885-1891, doi: 10.1017/s0950268812002609.

Bajer, A., Bednarska, M., Pawelczyk, A., Behnke, J. M., Gilbert, F. S. and Sinski, E. (2002). Prevalence and abundance of *Cryptosporidium parvum* and *Giardia* spp. in wild rural rodents from the Mazury Lake District region of Poland. Parasitology *125 (Pt 1)*, 21-34.

Bajer, A. (2008). Between-year variation and spatial dynamics of *Cryptosporidium* spp. and *Giardia* spp. infections in naturally infected rodent populations. Parasitology *135 (14)*, 1629-1649, doi: 10.1017/s0031182008004952.

Balčiauskas, L., Balčiauskienė, L. and Baltrūnaitė, L. (2010). Root vole, *Microtus oeconomus*, in Lithuania: changes in the distribution range. Folia Zoologica *59 (4)*, 267-278.

Balčiauskas, L., Balčiauskienė, L. and Janonytė, A. (2012). The influence of spring floods on small mammal communities in the Nemunas River Delta, Lithuania. Biologica *67 (6)*, 1220-1229.

Barbosa, S., Paupério, J., Pavlova, S. V., Alves, P. C. and Searle, J. B. (2018). The *Microtus* voles: Resolving the phylogeny of one of the most speciose mammalian genera using genomics. Mol Phylogenet Evol *125*, 85-92, doi: 10.1016/j.ympev.2018.03.017.

Benacer, D., Mohd Zain, S. N., Sim, S. Z., Mohd Khalid, M. K., Galloway, R. L., Souris, M. and Thong, K. L. (2016). Determination of *Leptospira borgpetersenii* serovar Javanica and *Leptospira interrogans* serovar Bataviae as the persistent *Leptospira* serovars circulating in the urban rat populations in Peninsular Malaysia. Parasit Vectors *9*, 117, doi: 10.1186/s13071-016-1400-1.

Bernshtein, A. D., Apekina, N. S., Mikhailova, T. V., Myasnikov, Y. A., Khlyap, L. A., Korotkov, Y. S. and Gavrilovskaya, I. N. (1999). Dynamics of Puumala hantavirus infection in naturally infected bank voles (Clethrinomys glareolus). Arch Virol *144 (12)*, 2415-2428.

Bharti, A. R., Nally, J. E., Ricaldi, J. N., Matthias, M. A., Diaz, M. M., Lovett, M. A., Levett, P. N., Gilman, R. H., Willig, M. R. and Gotuzzo, E. (2003). Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis *3 (12)*, 757-771.

Birnbaum, N., Barr, S. C., Center, S. A., Schermerhorn, T., Randolph, J. F. and Simpson, K. W. (1998). Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological features. J Small Anim Pract *39 (5)*, 231-236.

Blagojevic, J., Šekler, M., Rajičić, M., Pejić, B., Budinski, I., Jovanović, V. M., Adnadević, T., Vidanović, D., Matović, K. and Vujošević, M. (2019). The prevalence of pathogenic forms of *Leptospira* in natural populations of small wild mammals in Serbia. Acta Vet Hung *67 (3)*, 338-346, doi: 10.1556/004.2019.035.

Boot, R., Lammers, R. M. and Busschbach, A. E. (1986). Isolation of members of the Haemophilus-Pasteurella-Actinobacillus group from feral rodents. Lab Anim *20 (1)*, 36-40, doi: 10.1258/002367786781062124.

Bowen, M. D., Gelbmann, W., Ksiazek, T. G., Nichol, S. T. and Nowotny, N. (1997). Puumala virus and two genetic variants of Tula virus are present in Austrian rodents. J Med Virol *53 (2)*, 174-181, doi: 10.1002/(sici)1096-9071(199710)53:2<174::aid-jmv11>3.0.co;2-j.

Breitschwerdt, E. B. (2014). Bartonellosis: one health perspectives for an emerging infectious disease. Ilar j *55 (1)*, 46-58, doi: 10.1093/ilar/ilu015.

Brocato, R. L. and Hooper, J. W. (2019). Progress on the Prevention and Treatment of Hantavirus Disease. Viruses *11 (7)*, doi: 10.3390/v11070610.

Brummer-Korvenkontio, M., Vaheri, A., Hovi, T., von Bonsdorff, C. H., Vuorimies, J., Manni, T., Penttinen, K., Oker-Blom, N. and Lahdevirta, J. (1980). Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. J Infect Dis *141 (2)*, 131-134, doi: 10.1093/infdis/141.2.131.

Bryja, J., Nesvadbová, J., Heroldová, M., Jánová, E., Losík, J., Trebatická, L. and Tkadlec, E. (2005). Common vole (*Microtus arvalis*) population sex ratio: biases and process variation. Can J Zool *83 (11)*, 1391-1399.

Calderón, A., Rodríguez, V., Máttar, S. and Arrieta, G. (2014). Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. Trop Anim Health Prod *46 (2)*, 427-432.

Cann, K. F., Thomas, D. R., Salmon, R. L., Wyn-Jones, A. P. and Kay, D. (2013). Extreme water-related weather events and waterborne disease. Epidemiol Infect *141 (4)*, 671-686, doi: 10.1017/s0950268812001653.

Carey, D. E., Reuben, R., Panicker, K. N., Shope, R. E. and Myers, R. M. (1971). Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. Indian J Med Res *59 (11)*, 1758-1760.

Carroll, A. G. and Campbell, R. S. (1987). Reproductive and leptospiral studies on beef cattle in central Queensland. Aust Vet J *64 (1)*, 1-5, doi: 10.1111/j.1751-0813.1987.tb06046.x.

Carroll, D., Daszak, P., Wolfe, N. D., Gao, G. F., Morel, C. M., Morzaria, S., Pablos-Méndez, A., Tomori, O. and Mazet, J. A. K. (2018). The Global Virome Project. Science *359 (6378)*, 872-874, doi: 10.1126/science.aap7463.

Casanovas-Massana, A., Pedra, G. G., Wunder, E. A., Jr., Diggle, P. J., Begon, M. and Ko, A. I. (2018). Quantification of *Leptospira interrogans* Survival in Soil and Water Microcosms. Appl Environ Microbiol *84 (13)*, doi: 10.1128/aem.00507-18.

Castel, G., Chevenet, F., Razzauti, M., Murri, S., Marianneau, P., Cosson, J. F., Tordo, N. and Plyusnin, A. (2019). Phylogeography of Puumala orthohantavirus in Europe. Viruses *11 (8)*, doi: 10.3390/v11080679.

Castel, G., Couteaudier, M., Sauvage, F., Pons, J. B., Murri, S., Plyusnina, A., Pontier, D., Cosson, J. F., Plyusnin, A., Marianneau, P. and Tordo, N. (2015). Complete Genome and Phylogeny of Puumala Hantavirus Isolates Circulating in France. Viruses *7 (10)*, 5476-5488, doi: 10.3390/v7102884.

Chae, J. S., Yu, D. H., Shringi, S., Klein, T. A., Kim, H. C., Chong, S. T., Lee, I. Y. and Foley, J. (2008). Microbial pathogens in ticks, rodents and a shrew in northern Gyeonggi-do near the DMZ, Korea. J Vet Sci *9 (3)*, 285-293, doi: 10.4142/jvs.2008.9.3.285.

Chaline, J., Brunet-Lecomte, P. and Campy, M. (1995). The last glacial/interglacial record of rodent remains from the Gigny karst sequence in the French Jura used for palaeoclimatic and palaeoecological reconstructions. Palaeogeogr Palaeoclimatol Palaeoecol *117 (3-4)*, 229-252.

Chappell, J. G., Tsoleridis, T., Onianwa, O., Drake, G., Ashpole, I., Dobbs, P., Edema, W., Kumi-Ansah, F., Bennett, M., Tarlinton, R. E., Ball, J. K. and McClure, C. P. (2020). Retrieval of the complete coding sequence of the UK-endemic Tatenale orthohantavirus reveals extensive strain variation and supports its classification as a novel species. Viruses *12 (4)*, doi: 10.3390/v12040454.

Chen, J. T., Qin, J., Li, K., Xu, Q. Y., Wang, X. P., Plyusnin, A., Hou, W. and Zhang, Y. Z. (2019). Identification and characterization of a novel subtype of Tula virus in *Microtus arvalis obscurus* voles sampled from Xinjiang, China. Infect Genet Evol *75*, 104012, doi: 10.1016/j.meegid.2019.104012.

Chmel, L., Buchvald, J. and Valentova, M. (1975). SPREAD OF TRICHOPHYTON MENTAGROPHYTES VAR. GRAN. INFECTION TO MAN. Int J Dermatol *14 (4)*, 269-272.

Cislakova, L., Stanko, M., Fricova, J., Mosansky, L., Travnicek, M., Halanova, M., Mardzinova, S. and Stefancikova, A. (2004). Small mammals (Insectivora, Rodentia) as a potential source of chlamydial infection in East Slovakia. Ann Agric Environ Med *11 (1)*, 139-143.

Clement, J., Van Esbroeck, M., Lagrou, K., Verschueren, J., Sunil-Chandra, N. P. and Van Ranst, M. (2014). Leptospirosis versus hantavirus infections in the Netherlands and in Belgium, 2000 to 2014. Euro Surveill *19 (38)*, doi: 10.2807/1560-7917.es2014.19.38.20912.

Cortez, V., Canal, E., Dupont-Turkowsky, J. C., Quevedo, T., Albujar, C., Chang, T. C., Salmon-Mulanovich, G., Guezala-Villavicencio, M. C., Simons, M. P., Margolis, E., Schultz-Cherry, S., Pacheco, V. and Bausch, D. G. (2018). Identification of *Leptospira* and *Bartonella* among rodents collected across a habitat disturbance gradient along the Inter-Oceanic Highway in the southern Amazon Basin of Peru. PLoS One *13 (10)*, e0205068, doi: 10.1371/journal.pone.0205068.

Cosson, J.-F., Picardeau, M., Mielcarek, M., Tatard, C., Chaval, Y., Suputtamongkol, Y., Buchy, P., Jittapalapong, S., Herbreteau, V. and Morand, S. (2014). Epidemiology of *Leptospira* Transmitted by Rodents in Southeast Asia. PLoS Negl Trop Dis *8 (6)*, e2902.

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., Stein, C., Abela-Ridder, B. and Ko, A. I. (2015). Global morbidity and mortality of leptospirosis: a systematic review. PLoS Negl Trop Dis *9 (9)*, e0003898.

Costa, F., Porter, F. H., Rodrigues, G., Farias, H., de Faria, M. T., Wunder, E. A., Osikowicz, L. M., Kosoy, M. Y., Reis, M. G., Ko, A. I. and Childs, J. E. (2014). Infections by *Leptospira interrogans*, Seoul virus, and *Bartonella* spp. among Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil. Vector Borne Zoonotic Dis *14 (1)*, 33-40, doi: 10.1089/vbz.2013.1378.

Cutler, S., Vayssier-Taussat, M., Estrada-Peña, A., Potkonjak, A., Mihalca, A. D. and Zeller, H. (2019). A new Borrelia on the block: Borrelia miyamotoi - a human health risk? Euro Surveill *24 (18)*, doi: 10.2807/1560-7917.Es.2019.24.18.1800170.

Cvetko, L., Turk, N., Markotić, A., Milas, Z., Margaletić, J., Miletić-Medved, M., Plyusnin, A., Baranton, G., Postic, D. and Avsic-Zupanc, T. (2006). Short report: dual infections with *Puumala* virus and *Leptospira interrogans* serovar lora in a bank vole (*Clethrionomys glareolus*). Am J Trop Med Hyg *74 (4)*, 612-614.

Dabrowski, M. J., Pomorski, J. J. and Gliwicz, J. (2013). Cytochrome b gene (*cytb*) sequence diversity in a *Microtus oeconomus* population from Bialowieza Primeval Forest. Acta Theriol (Warsz) *58 (2)*, 119-126, doi: 10.1007/s13364-012-0096-7.

Desai, S., van Treeck, U., Lierz, M., Espelage, W., Zota, L., Sarbu, A., Czerwinski, M., Sadkowska-Todys, M., Avdicova, M., Reetz, J., Luge, E., Guerra, B., Nockler, K. and Jansen, A. (2009). Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. Clin Infect Dis *48 (6)*, 691-697, doi: 10.1086/597036.

Desvars-Larrive, A., Pascal, M., Gasqui, P., Cosson, J. F., Benoit, E., Lattard, V., Crespin, L., Lorvelec, O., Pisanu, B., Teynie, A., Vayssier-Taussat, M., Bonnet, S., Marianneau, P., Lacote, S., Bourhy, P., Berny, P., Pavio, N., Le Poder, S., Gilot-Fromont, E., Jourdain, E., Hammed, A., Fourel, I., Chikh, F. and Vourc'h, G. (2017). Population genetics, community of parasites, and resistance to rodenticides in an urban brown rat (*Rattus norvegicus*) population. PLoS One *12 (9)*, e0184015, doi: 10.1371/journal.pone.0184015.

Deter, J., Chaval, Y., Galan, M., Gauffre, B., Morand, S., Henttonen, H., Laakkonen, J., Voutilainen, L., Charbonnel, N. and Cosson, J. F. (2008). Kinship, dispersal and hantavirus transmission in bank and common voles. Arch Virol *153 (3)*, 435-444, doi: 10.1007/s00705-007-0005-6.

Diaz, J. H. (2015). Rodent-borne infectious disease outbreaks after flooding disasters: Epidemiology, management, and prevention. Am J Disaster Med *10 (3)*, 259-267, doi: 10.5055/ajdm.2015.0207.

Dietrich, M., Muhldorfer, K., Tortosa, P. and Markotter, W. (2015). *Leptospira* and Bats: Story of an Emerging Friendship. PLoS Pathog *11 (11)*, e1005176, doi: 10.1371/journal.ppat.1005176.

Dreesman, J., Hamschmidt, L., Toikkanen, S., Runge, M., Lüsse, B., Freise, J., Ehlers, J., Nöckler, K., Knorr, C. and Keller, B. (2016). Leptospirose-Ausbruch bei Saisonarbeitern in der Erdbeerernte in Niedersachsen, 2014. Das Gesundheitswesen *78 (04)*, V77.

Drewes, S., Ali, H. S., Saxenhofer, M., Rosenfeld, U. M., Binder, F., Cuypers, F., Schlegel, M., Röhrs, S., Heckel, G. and Ulrich, R. G. (2017a). Host-associated absence of human Puumala virus infections in northern and eastern Germany. Emerg Infect Dis *23 (1)*, 83-86, doi: 10.3201/eid2301.160224.

Drewes, S., Turni, H., Rosenfeld, U. M., Obiegala, A., Straková, P., Imholt, C., Glatthaar, E., Dressel, K., Pfeffer, M., Jacob, J., Wagner-Wiening, C. and Ulrich, R. G. (2017b). Reservoir-Driven Heterogeneous Distribution of Recorded Human Puumala virus Cases in South-West Germany. Zoonoses Public Health *64 (5)*, 381-390, doi: 10.1111/zph.12319.

Dzagurova, T. K., Witkowski, P. T., Tkachenko, E. A., Klempa, B., Morozov, V. G., Auste, B., Zavora, D. L., Iunicheva, I. V., Mutnih, E. S. and Kruger, D. H. (2012). Isolation of Sochi Virus From a Fatal Case of Hantavirus Disease With Fulminant Clinical Course. Clin Infect Dis *54 (1)*, e1-4, doi: 10.1093/cid/cir746.

Elashvili, E., Kracalik, I., Burjanadze, I., Datukishvili, S., Chanturia, G., Tsertsvadze, N., Beridze, L., Shavishvili, M., Dzneladze, A., Grdzelidze, M., Imnadze, P., Pearson, A. and Blackburn, J. K. (2015). Environmental Monitoring and Surveillance of Rodents and Vectors for *Francisella tularensis* Following Outbreaks of Human Tularemia in Georgia. Vector Borne Zoonotic Dis *15 (10)*, 633-636, doi: 10.1089/vbz.2015.1781.

Ermonval, M., Baychelier, F. and Tordo, N. (2016). What Do We Know about How Hantaviruses Interact with Their Different Hosts? Viruses *8 (8)*, doi: 10.3390/v8080223.

Escutenaire, S., Chalon, P., De Jaegere, F., Karelle-Bui, L., Mees, G., Brochier, B., Rozenfeld, F. and Pastoret, P. P. (2002). Behavioral, physiologic, and habitat influences on the dynamics of *Puumala virus* infection in bank voles (*Clethrionomys glareolus*). Emerg Infect Dis *8 (9)*, 930-936, doi: 10.3201/eid0809.010537.

Essbauer, S., Schmidt, J., Conraths, F. J., Friedrich, R., Koch, J., Hautmann, W., Pfeffer, M., Wolfel, R., Finke, J., Dobler, G. and Ulrich, R. (2006). A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. Epidemiol Infect *134 (6)*, 1333-1344, doi: 10.1017/s095026 8806006170.

Essbauer, S. S., Schmidt-Chanasit, J., Madeja, E. L., Wegener, W., Friedrich, R., Petraityte, R., Sasnauskas, K., Jacob, J., Koch, J., Dobler, G., Conraths, F. J., Pfeffer, M., Pitra, C. and Ulrich, R. G. (2007). Nephropathia epidemica in metropolitan area, Germany. Emerg Infect Dis *13 (8)*, 1271-1273, doi: 10.3201/eid1308.061425.

Faber, M., Krüger, D. H., Auste, B., Stark, K., Hofmann, J. and Weiss, S. (2019). Molecular and epidemiological characteristics of human Puumala and Dobrava-Belgrade hantavirus infections, Germany, 2001 to 2017. Euro Surveill *24 (32)*, doi: 10.2807/1560-7917.es.2019.24.32.1800675.

Faber, M., Wollny, T., Schlegel, M., Wanka, K. M., Thiel, J., Frank, C., Rimek, D., Ulrich, R. G. and Stark, K. (2013). Puumala virus outbreak in Western Thuringia, Germany, 2010: epidemiology and strain identification. Zoonoses Public Health *60 (8)*, 549-554, doi: 10.1111/zph.12037.

Faine, S., Adler, B., Bolin, C. and Perolat, P. (1999). Leptospira and leptospirosis, MediSci. Melbourne, Australia.

Feher, E., Kemenesi, G., Oldal, M., Kurucz, K., Kugler, R., Farkas, S. L., Marton, S., Horvath, G., Banyai, K. and Jakab, F. (2017). Isolation and complete genome characterization of novel reassortant orthoreovirus from common vole (*Microtus arvalis*). Virus Genes *53 (2)*, 307-311, doi: 10.1007/s11262-016-1411-1.

Feliu, C., Spakulova, M., Casanova, J. C., Renaud, F., Morand, S., Hugot, J. P., Santalla, F. and Durand, P. (2000). GENETIC AND MORPHOLOGICAL HETEROGENEITY IN SMALL RODENT WHIPWORMS IN SOUTHWESTERN EUROPE: CHARACTE-RIZATION OF *TRICHURIS MURIS* AND DESCRIPTION OF *TRICHURIS ARVICOLAE* N. SP. (NEMATODA: TRICHURIDAE). J Parasitol *86 (3)*, 442-449, doi: 10.1645/0022-3395(2000)086[0442:gamhis]2.0.co;2.

Fichet-Calvet, E., Kia, E. B., Giraudoux, P., Quere, J. P., Delattre, P. and Ashford, R. W. (2004). Frenkelia parasites in a small mammal community. Dynamics of infection and effect on the host. Parasite *11 (3)*, 301-310.

Fischer, O., Matlova, L., Bartl, J., Dvorska, L., Melicharek, I. and Pavlik, I. (2000). Findings of mycobacteria in insectivores and small rodents. Folia Microbiol (Praha) *45 (2)*, 147-152.

Fischer, S., Mayer-Scholl, A., Imholt, C., Spierling, N. G., Heuser, E., Schmidt, S., Reil, D., Rosenfeld, U. M., Jacob, J., Nockler, K. and Ulrich, R. G. (2018a). *Leptospira* Genomospecies and Sequence Type Prevalence in Small Mammal Populations in Germany. Vector Borne Zoonotic Dis *18 (4)*, 188-199, doi: 10.1089/vbz.2017.2140.

Fischer, S., Spierling, N. G., Heuser, E., Kling, C., Schmidt, S., Rosenfeld, U. M., Reil, D., Imholt, C., Jacob, J., Ulrich, R. G. and Essbauer, S. (2018b). High prevalence of *Rickettsia helvetica* in wild small mammal populations in Germany. Ticks Tick Borne Dis, doi: 10.1016/j.ttbdis.2018.01.009.

Fletcher, N. K., Acevedo, P., Herman, J. S., Paupério, J., Alves, P. C. and Searle, J. B. (2019). Glacial cycles drive rapid divergence of cryptic field vole species. Ecol Evol *9 (24)*, 14101-14113, doi: 10.1002/ece3.5846.

Fornazari, F. (2017). Are Reptiles Reservoirs of Leptospirosis? A Brief Discussion Based on Serological Studies. Ecohealth *14 (2)*, 203-204, doi: 10.1007/s10393-017-1243-z.

Fraga, T. R., Isaac, L. and Barbosa, A. S. (2016). Complement Evasion by Pathogenic *Leptospira*. Front Immunol *7*, 623, doi: 10.3389/fimmu.2016.00623.

Friedman, M., Bednar, V., Klimes, J., Smola, J., Mrlik, V. and Literak, I. (2008). *Lawsonia intracellularis* in rodents from pig farms with the occurrence of porcine proliferative enteropathy. Lett Appl Microbiol *47 (2)*, 117-121, doi: 10.1111/j.1472-765X.2008.02394.x.

Führer, H. P., Bloschl, I., Siehs, C. and Hassl, A. (2010a). Detection of *Toxoplasma gondii*, *Neospora caninum*, and *Encephalitozoon cuniculi* in the brains of common voles (*Microtus arvalis*) and water voles (*Arvicola terrestris*) by gene amplification techniques in western Austria (Vorarlberg). Parasitol Res *107 (2)*, 469-473, doi: 10.1007/s00436-010-1905-z.

Führer, H. P., Schneider, R., Walochnik, J. and Auer, H. (2010b). Extraintestinal helminths of the common vole (*Microtus arvalis*) and the water vole (*Arvicola terrestris*) in Western Austria (Vorarlberg). Parasitol Res *106 (4)*, 1001-1004, doi: 10.1007/s00436-010-1753-x.

Gamage, C. D., Sato, Y., Kimura, R., Yamashiro, T. and Toma, C. (2020). Understanding leptospirosis eco-epidemiology by environmental DNA metabarcoding of irrigation water from two agro-ecological regions of Sri Lanka. PLoS Negl Trop Dis *14 (7)*, e0008437, doi: 10.1371/journal.pntd.0008437.

García, J. T., Domínguez-Villaseñor, J., Alda, F., Calero-Riestra, M., Pérez Olea, P., Fargallo, J. A., Martínez-Padilla, J., Herranz, J., Oñate, J. J. and Santamaría, A. (2020). A complex scenario of glacial survival in Mediterranean and continental refugia of a temperate continental vole species (*Microtus arvalis*) in Europe. J Zoolog Syst Evol Res *58 (1)*, 459-474, doi: https://doi.org/10.1111/jzs.12323.

Gavrilovskaya, I. N., Brown, E. J., Ginsberg, M. H. and Mackow, E. R. (1999). Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J Virol *73 (5)*, 3951-3959.

Gavrilovskaya, I. N., Shepley, M., Shaw, R., Ginsberg, M. H. and Mackow, E. R. (1998). beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A *95 (12)*, 7074-7079, doi: 10.1073/pnas.95.12.7074.

Geimonen, E., Neff, S., Raymond, T., Kocer, S. S., Gavrilovskaya, I. N. and Mackow, E. R. (2002). Pathogenic and nonpathogenic hantaviruses differentially regulate endothelial cell responses. Proc Natl Acad Sci USA *99 (21)*, 13837-13842, doi: 10.1073/pnas.192298899.

Geoghegan, J. L., Senior, A. M., Di Giallonardo, F. and Holmes, E. C. (2016). Virological factors that increase the transmissibility of emerging human viruses. Proc Natl Acad Sci U S A *113 (15)*, 4170-4175, doi: 10.1073/pnas.1521582113.

Glatz, M., Müllegger, R. R., Maurer, F., Fingerle, V., Achermann, Y., Wilske, B. and Bloemberg, G. V. (2014). Detection of *Candidatus* Neoehrlichia mikurensis, *Borrelia burgdorferi* sensu lato genospecies and *Anaplasma phagocytophilum* in a tick population from Austria. Ticks Tick Borne Dis *5 (2)*, 139-144, doi: 10.1016/j.ttbdis.2013.10.006.

Gligic, A., Dimkovic, N., Xiao, S. Y., Buckle, G. J., Jovanovic, D., Velimirovic, D., Stojanovic, R., Obradovic, M., Diglisic, G., Micic, J. and et al. (1992). Belgrade virus: a new hantavirus causing severe hemorrhagic fever with renal syndrome in Yugoslavia. J Infect Dis *166 (1)*, 113-120, doi: 10.1093/infdis/166.1.113.

Goeijenbier, M., Hartskeerl, R. A., Reimerink, J., Verner-Carlsson, J., Wagenaar, J. F., Goris, M. G., Martina, B. E., Lundkvist, A., Koopmans, M., Osterhaus, A. D., van Gorp, E. C. and Reusken, C. B. (2014). The hanta hunting study: underdiagnosis of Puumala hantavirus infections in symptomatic non-travelling leptospirosis-suspected patients in the Netherlands, in 2010 and April to November 2011. Euro Surveill *19 (32)*, doi: 10.2807/1560-7917.es2014.19.32.20878.

Golovljova, I., Vasilenko, V., Mittženkov, V., Prükk, T., Seppet, E., Vene, S., Settergren, B., Plyusnin, A. and Lundkvist, Å. (2007). Characterization of hemorrhagic fever with renal syndrome caused by hantaviruses, Estonia. Emerg Infect Dis *13 (11)*, 1773, doi: 10.3201/eid1311.070221.

Gottstein, B., Saucy, F., Deplazes, P., Reichen, J., Demierre, G., Busato, A., Zuercher, C. and Pugin, P. (2001). Is high prevalence of Echinococcus multilocularis in wild and domestic animals associated with disease incidence in humans? Emerg Infect Dis *7 (3)*, 408-412, doi: 10.3201/eid0703.010307.

Gouveia, E. L., Metcalfe, J., de Carvalho, A. L., Aires, T. S., Villasboas-Bisneto, J. C., Queirroz, A., Santos, A. C., Salgado, K., Reis, M. G. and Ko, A. I. (2008). Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil. Emerg Infect Dis *14 (3)*, 505-508, doi: 10.3201/eid1403.071064.

Graham, A. L. (2008). Ecological rules governing helminth-microparasite coinfection. Proc Natl Acad Sci U S A *105 (2)*, 566-570, doi: 10.1073/pnas.0707221105.

Gryczyńska, A., Gortat, T. and Kowalec, M. (2018). Urban rodent reservoirs of *Borrelia* spp. in Warsaw, Poland. Epidemiol Infect *146 (5)*, 589-593, doi: 10.1017/s0950 26881800033x.

Gu, S. H., Hejduk, J., Markowski, J., Kang, H. J., Markowski, M., Połatyńska, M., Sikorska, B., Liberski, P. P. and Yanagihara, R. (2014). Co-circulation of soricid- and talpid-borne hantaviruses in Poland. Infect Genet Evol *28*, 296-303, doi: 10.1016/j.meegid.2014.10.017.

Guivier, E., Galan, M., Henttonen, H., Cosson, J. F. and Charbonnel, N. (2014). Landscape features and helminth co-infection shape bank vole immunoheterogeneity, with consequences for Puumala virus epidemiology. Heredity (Edinb) *112 (3)*, 274-281, doi: 10.1038/hdy.2013.103.

Guo, G., Guo, B., Wu, X., Wang, Y., Bao, J., Ren, Y., Li, H., Li, J., Zhang, W. and Yao, H. (2019). Molecular evidence of Tula virus in *Microtus obscurus* in the region of Yili, Xinjiang, China. BMC Infect Dis *19 (1)*, 527, doi: 10.1186/s12879-019-4133-x.

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I. S. and Harrus, S. (2015). *Bartonella* infection in rodents and their flea ectoparasites: an overview. Vector Borne Zoonotic Dis *15 (1)*, 27-39, doi: 10.1089/vbz.2014.1606.

Hallmaier-Wacker, L. K., Munster, V. J. and Knauf, S. (2017). Disease reservoirs: from conceptual frameworks to applicable criteria. Emerg Microbes Infect *6 (9)*, e79, doi: 10.1038/emi.2017.65.

Hamsikova, Z., Coipan, C., Mahrikova, L., Minichova, L., Sprong, H. and Kazimirova, M. (2016). *Borrelia miyamotoi* and Co-Infection with *Borrelia afzelii* in *Ixodes ricinus* Ticks and Rodents from Slovakia. Microb Ecol, doi: 10.1007/s00248-016-0918-2.

Han, B. A., Schmidt, J. P., Bowden, S. E. and Drake, J. M. (2015). Rodent reservoirs of future zoonotic diseases. Proc Natl Acad Sci USA *112 (22)*, 7039-7044, doi: 10.1073/pnas.1501598112.

Hanosset, R., Saegerman, C., Adant, S., Massart, L. and Losson, B. (2008). *Echinococcus multilocularis* in Belgium: prevalence in red foxes (*Vulpes vulpes*) and in different species of potential intermediate hosts. Vet Parasitol *151 (2-4)*, 212-217, doi: 10.1016/j.vetpar.2007.09.024.

Hardestam, J., Karlsson, M., Falk, K. I., Olsson, G., Klingström, J. and Lundkvist, Ä. (2008). Puumala hantavirus excretion kinetics in bank voles (*Myodes glareolus*). Emerg Infect Dis *14 (8)*, 1209-1215, doi: 10.3201/eid1408.080221.

Hassell, J. M., Begon, M., Ward, M. J. and Fevre, E. M. (2017). Urbanization and Disease Emergence: Dynamics at the Wildlife-Livestock-Human Interface. Trends Ecol Evol *32 (1)*, 55-67, doi: 10.1016/j.tree.2016.09.012.

Haydon, D. T., Cleaveland, S., Taylor, L. H. and Laurenson, M. K. (2002). Identifying reservoirs of infection: a conceptual and practical challenge. Emerg Infect Dis *8 (12)*, 1468-1473, doi: 10.3201/eid0812.010317.

Heckel, G., Burri, R., Fink, S., Desmet, J. F. and Excoffier, L. (2005). Genetic structure and colonization processes in European populations of the common vole, *Microtus arvalis*. Evolution *59 (10)*, 2231-2242, doi: 10.1111/j.0014-3820.2005.tb00931.x.

Heglasova, I., Vichova, B., Kraljik, J., Mosansky, L., Miklisova, D. and Stanko, M. (2018). Molecular evidence and diversity of the spotted-fever group *Rickettsia* spp. in small mammals from natural, suburban and urban areas of Eastern Slovakia. Ticks Tick Borne Dis *9 (6)*, 1400-1406, doi: 10.1016/j.ttbdis.2018.06.011.

Helmy, Y. A., Spierling, N. G., Schmidt, S., Rosenfeld, U. M., Reil, D., Imholt, C., Jacob, J., Ulrich, R. G., Aebischer, T. and Klotz, C. (2018). Occurrence and distribution of *Giardia* species in wild rodents in Germany. Parasit Vectors *11 (1)*, 213, doi: 10.1186/s13071-018-2802-z.

Herbreteau, V., Bordes, F., Jittapalapong, S., Supputamongkol, Y. and Morand, S. (2012). Rodent-borne diseases in Thailand: targeting rodent carriers and risky habitats. Infect Ecol Epidemiol *2*, doi: 10.3402/iee.v2i0.18637.

Herman, J. S., McDevitt, A. D., Kawalko, A., Jaarola, M., Wojcik, J. M. and Searle, J. B. (2014). Land-Bridge Calibration of Molecular Clocks and the Post-Glacial Colonization of Scandinavia by the Eurasian Field Vole *Microtus agrestis*. PLoS One *9 (8)*, e103949, doi: 10.1371/journal.pone.0103949.

Herman, J. S. and Searle, J. B. (2011). Post-glacial partitioning of mitochondrial genetic variation in the field vole. Proc Biol Sci *278 (1724)*, 3601-3607, doi: 10.1098/rspb.2011.0321.

Hermannsen, J. (1954). Über eine Feldfieberepidemie im Westküstengebiet Schleswig-Holsteins. Dtsch Med Wochenschr *79 (7)*, 245.

Heuser, E., Fischer, S., Ryll, R., Mayer-Scholl, A., Hoffmann, D., Spahr, C., Imholt, C., Alfa, D. M., Frohlich, A., Luschow, D., Johne, R., Ehlers, B., Essbauer, S., Nockler, K. and Ulrich, R. G. (2017). Survey for zoonotic pathogens in Norway rat populations from Europe. Pest Manag Sci *73 (2)*, 341-348, doi: 10.1002/ps.4339.

Heyman, P., Klingström, J., de Jaegere, F., Leclercq, G., Rozenfeld, F., Escutenaire, S., Vandenvelde, C., Zizi, M., Plyusnin, A. and Lundkvist, Ä. (2002). Tula hantavirus in Belgium. Epidemiol Infect *128 (2)*, 251-256.

Heyman, P., Vaheri, A., Lundkvist, Ä. and Avsic-Zupanc, T. (2009). Hantavirus infections in Europe: from virus carriers to a major public-health problem. Expert Rev Anti Infect Ther *7 (2)*, 205-217, doi: 10.1586/14787210.7.2.205.

Hildebrand, J., Paziewska-Harris, A., Zalesny, G. and Harris, P. D. (2013). PCR characterization suggests that an unusual range of *Bartonella* species infect the striped field mouse (*Apodemus agrarius*) in Central Europe. Appl Environ Microbiol *79 (16)*, 5082-5084, doi: 10.1128/aem.01013-13.

Hjelle, B. and Yates, T. (2001). Modeling hantavirus maintenance and transmission in rodent communities. In: Hantaviruses, eds. Schmaljohn, C. S. and Nichol, S. T., Springer, Berlin, Germany, pp. 77-90.

Hofmann, J., Meier, M., Enders, M., Führer, A., Ettinger, J., Klempa, B., Schmidt, S., Ulrich, R. G. and Krüger, D. H. (2014). Hantavirus disease in Germany due to infection with Dobrava-Belgrade virus genotype Kurkino. Clin Microbiol Infect *20 (10)*, O648-655, doi: 10.1111/1469-0691.12543.

Hong, S. H., Lee, S. E., Jeong, Y. I., Kim, H. C., Chong, S. T., Klein, T. A., Song, J. W., Gu, S. H., Cho, S. H. and Lee, W. J. (2014). Prevalence and molecular characterizations of *Toxoplasma gondii* and *Babesia microti* from small mammals captured in Gyeonggi and Gangwon Provinces, Republic of Korea. Vet Parasitol *205 (3-4)*, 512-517, doi: 10.1016/j.vetpar.2014.07.032.

Hoogenboom, I. and Dijkstra, C. (1987). *Sarcocystis cernae*: a parasite increasing the risk of predation of its intermediate host, *Microtus arvalis*. Oecologia *74 (1)*, 86-92.

Hubalek, Z., Nesvadbova, J. and Rychnovsky, B. (1995). A heterogeneous distribution of *Emmonsia parva* var. *crescens* in an agro-ecosystem. J Med Vet Mycol *33 (3)*, 197-200.

Hubalek, Z., Pacova, Z., Halouzka, J., Sedlacek, I., Dlouhy, M. and Honza, M. (1998). Selective isolation of *Pseudomonas stutzeri* from vertebrate faeces on Rambach agar. Zentralbl Bakteriol *288 (3)*, 343-349.

Hubalek, Z. (1999). Emmonsiosis of wild rodents and insectivores in Czechland. J Wildl Dis *35 (2)*, 243-249, doi: 10.7589/0090-3558-35.2.243.

Hubalek, Z., Scholz, H. C., Sedlacek, I., Melzer, F., Sanogo, Y. O. and Nesvadbova, J. (2007). Brucellosis of the common vole (*Microtus arvalis*). Vector Borne Zoonotic Dis *7 (4)*, 679-687, doi: 10.1089/vbz.2007.0143.

Hurd, J., Berke, O., Poljak, Z. and Runge, M. (2017). Spatial analysis of *Leptospira* infection in muskrats in Lower Saxony, Germany, and the association with human leptospirosis. Res Vet Sci *114*, 351-354, doi: 10.1016/j.rvsc.2017.06.015.

Hutterer, R., Kryštufek, B., Yigit, N., Mitsain, G., Palomo, L., Henttonen, H., Vohralík, V., Zagorodnyuk, I., Juškaitis, R., Meinig, H. and Bertolino, S. (2016). *Myodes glareolus* (errata version published in 2017). URL: https://www.iucnredlist.org/species/4973/115070929 [as of 24.05.2020].

International Committee on Taxonomy of Viruses (2016). URL: https://talk.ictvonline.org/ICTV/proposals/2016.023a-cM.A.v2.Hantavirus_sprev.pdf [as of 15.05.2020].

International Committee on Taxonomy of Viruses (2020). URL: https://talk.ictvonline.org/taxonomy/w/ictv-taxonomy. [as of 15.05.2020].

Ivanauskas, T., Likevičienė, N. and Maldžiūnaitė, S. (1964). [Guidebook of Lithuanian mammals] [in Lithuanian], Valstybinė politinės ir mokslinės literatūros leidykla, Vilnius.

Ivanova, S., Herbreteau, V., Blasdell, K., Chaval, Y., Buchy, P., Guillard, B. and Morand, S. (2012). *Leptospira* and rodents in Cambodia: environmental determinants of infection. Am J Trop Med Hyg *86 (6)*, 1032-1038, doi: 10.4269/ajtmh.2012.11-0349.

Jaarola, M. and Searle, J. B. (2002). Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. Mol Ecol *11 (12)*, 2613-2621, doi: 10.1046/j.1365-294x.2002.01639.x.

Jääskelainen, K. M., Kaukinen, P., Minskaya, E. S., Plyusnina, A., Vapalahti, O., Elliott, R. M., Weber, F., Vaheri, A. and Plyusnin, A. (2007). Tula and Puumala hantavirus NSs ORFs are functional and the products inhibit activation of the interferon-beta promoter. J Med Virol *79 (10)*, 1527-1536, doi: 10.1002/jmv.20948.

Jääskeläinen, K. M., Plyusnina, A., Lundkvist, A., Vaheri, A. and Plyusnin, A. (2008). Tula hantavirus isolate with the full-length ORF for nonstructural protein NSs survives for more consequent passages in interferon-competent cells than the isolate having truncated NSs ORF. Virol J *5*, 3, doi: 10.1186/1743-422x-5-3.

Jackson, J. A., Begon, M., Birtles, R., Paterson, S., Friberg, I. M., Hall, A., Lowe, A., Ralli, C., Turner, A., Zawadzka, M. and Bradley, J. E. (2011). The analysis of immunological profiles in wild animals: a case study on immunodynamics in the field vole, *Microtus agrestis*. Mol Ecol *20 (5)*, 893-909, doi: 10.1111/j.1365-294X.2010.04907.x.

Jakab, F., Horvath, G., Ferenczi, E., Sebok, J. and Szucs, G. (2008). First detection of Tula hantaviruses in *Microtus arvalis* voles in Hungary. Arch Virol *153 (11)*, 2093-2096, doi: 10.1007/s00705-008-0216-5.

Jakab, F., Horvath, G., Ferenczi, E., Sebok, J., Varecza, Z. and Szucs, G. (2007a). Detection of Dobrava hantaviruses in *Apodemus agrarius* mice in the Transdanubian region of Hungary. Virus Res *128 (1-2)*, 149-152, doi: 10.1016/j.virusres.2007.04.015.

Jakab, F., Sebok, J., Ferenczi, E., Horvath, G. and Szucs, G. (2007b). First detection of Dobrava hantavirus from a patient with severe haemorrhagic fever with renal syndrome by SYBR Green-based real time RT-PCR. Scand J Infect Dis *39 (10)*, 902-906, doi: 10.1080/00365540701387072.

Janova, E., Skoric, M., Heroldova, M., Tenora, F., Fictum, P. and Pavlik, I. (2010). Determinants of the prevalence of *Heligmosomum costellatum* (Heligmosomidae: Trichostrongyloidea) in a common vole population in southern Moravia, Czech Republic. J Helminthol *84 (4)*, 410-414, doi: 10.1017/s0022149x1000009x.

Jeske, K., Tomaso, H., Imholt, C., Schulz, J., Beerli, O., Suchomel, J., Heroldova, M., Jacob, J., Staubach, C. and Ulrich, R. G. (2019a). Detection of *Francisella tularensis* in three vole species in Central Europe. Transbound Emerg Dis *66 (2)*, 1029-1032, doi: 10.1111/tbed.13078.

Jeske, K., Weber, S., Pfaff, F., Imholt, C., Jacob, J., Beer, M., Ulrich, R. G. and Hoffmann, D. J. V. (2019b). Molecular Detection and Characterization of the First Cowpox Virus Isolate Derived from a Bank Vole. Viruses *11 (11)*, 1075, doi: 10.3390/v11111075.

Jha, P., Kim, C. M., Kim, D. M., Yoon, N. R., Jha, B., Park, J. W. and Chung, J. K. (2018). First detection and identification of *Candidatus* Neoehrlichia mikurensis in South Korea. PLoS One *13 (12)*, e0209685, doi: 10.1371/journal.pone.0209685.

Jiang, H., Du, H., Wang, L. M., Wang, P. Z. and Bai, X. F. (2016). Hemorrhagic Fever with Renal Syndrome: Pathogenesis and Clinical Picture. Front Cell Infect Microbiol *6*, 1, doi: 10.3389/fcimb.2016.00001.

Jobbins, S. E. and Alexander, K. A. (2015). Evidence of *Leptospira* sp. infection among a diversity of African wildlife species: beyond the usual suspects. Trans R Soc Trop Med Hyg *109 (5)*, 349-351, doi: 10.1093/trstmh/trv007.

Johnson, P. T., Ostfeld, R. S. and Keesing, F. (2015). Frontiers in research on biodiversity and disease. Ecol Lett *18 (10)*, 1119-1133, doi: 10.1111/ele.12479.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L. and Daszak, P. (2008). Global trends in emerging infectious diseases. Nature *451 (7181)*, 990.

Jonsson, C. B., Figueiredo, L. T. and Vapalahti, O. (2010). A global perspective on hantavirus ecology, epidemiology, and disease. Clin Microbiol Rev *23 (2)*, 412-441, doi: 10.1128/cmr.00062-09.

Kallio, E. R., Begon, M., Henttonen, H., Koskela, E., Mappes, T., Vaheri, A. and Vapalahti, O. (2010). Hantavirus infections in fluctuating host populations: the role of maternal antibodies. Proc Biol Sci *277 (1701)*, 3783-3791, doi: 10.1098/rspb.2010.1022.

Kallio, E. R., Klingström, J., Gustafsson, E., Manni, T., Vaheri, A., Henttonen, H., Vapalahti, O. and Lundkvist, Ä. (2006a). Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. J Gen Virol *87 (Pt 8)*, 2127-2134, doi: 10.1099/vir.0.81643-0.

Kallio, E. R., Poikonen, A., Vaheri, A., Vapalahti, O., Henttonen, H., Koskela, E. and Mappes, T. (2006b). Maternal antibodies postpone hantavirus infection and enhance individual breeding success. Proc Biol Sci *273 (1602)*, 2771-2776, doi: 10.1098/rspb.2006.3645.

Kallio, E. R., Voutilainen, L., Vapalahti, O., Vaheri, A., Henttonen, H., Koskela, E. and Mappes, T. (2007). Endemic hantavirus infection impairs the winter survival of its rodent host. Ecology *88 (8)*, 1911-1916, doi: 10.1890/06-1620.1

Kallio-Kokko, H., Laakkonen, J., Rizzoli, A., Tagliapietra, V., Cattadori, I., Perkins, S. E., Hudson, P. J., Cristofolini, A., Versini, W., Vapalahti, O., Vaheri, A. and Henttonen, H. (2006). Hantavirus and arenavirus antibody prevalence in rodents and humans in Trentino, Northern Italy. Epidemiol Infect *134 (4)*, 830-836, doi: 10.1017/s0950268805005431.

Kamal, S. M. and El Sayed Khalifa, K. (2006). Immune modulation by helminthic infections: worms and viral infections. Parasite Immunol *28 (10)*, 483-496, doi: 10.1111/j.1365-3024.2006.00909.x.

Kaneko, Y., Kryštufek, B., Zagarondnyuk, I., Vohralík, V., Batsaikhan, N., Avirmed, D. and Sukhchuluun, G. (2016). *Apodemus agrarius* (errata version published in 2017). URL: https://www.iucnredlist.org/species/1888/115057408 [as of 24.05.2020].

Kang, H. J., Bennett, S. N., Dizney, L., Sumibcay, L., Arai, S., Ruedas, L. A., Song, J. W. and Yanagihara, R. (2009). Host switch during evolution of a genetically distinct hantavirus in the American shrew mole (*Neurotrichus gibbsii*). Virology *388 (1)*, 8-14, doi: 10.1016/j.virol.2009.03.019.

Kariwa, H., Tkachenko, E. A., Morozov, V. G., Seto, T., Tanikawa, Y., Kolominov, S. I., Belov, S. N., Nakamura, I., Hashimoto, N., Balakiev, A. E., Dzagurnova, T. K., Daud, N. H., Miyashita, D., Medvedkina, O. A., Nakauchi, M., Ishizuka, M., Yoshii, K., Yoshimatsu, K., Arikawa, J. and Takashima, I. (2009). Epidemiological study of hantavirus infection in the Samara Region of European Russia. J Vet Med Sci *71 (12)*, 1569-1578, doi: 10.1292/jvms.001569.

Kariwa, H., Yoshizumi, S., Arikawa, J., Yoshimatsu, K., Takahashi, K., Takashima, I. and Hashimoto, N. (1995). Evidence for the existence of Puumula-related virus among *Clethrionomys rufocanus* in Hokkaido, Japan. Am J Trop Med Hyg *53 (3)*, 222-227, doi: 10.4269/ajtmh.1995.53.222.

Kaya, S., Yilmaz, G., Aydin, M., Aksoy, F. and Koksal, I. (2019). The "MICE" scoring system in differentiating the identical twins leptospirosis and hantavirus infection. Infection, doi: 10.1007/s15010-019-01366-7.

Kaysser, P., Seibold, E., Matz-Rensing, K., Pfeffer, M., Essbauer, S. and Splettstoesser, W. D. (2008). Re-emergence of tularemia in Germany: presence of *Francisella tularensis* in different rodent species in endemic areas. BMC Infect Dis *8*, 157, doi: 10.1186/1471-2334-8-157.

Khairani-Bejo, S., Bahaman, A., Zamri-Saad, M. and Mutalib, A. (2004). The survival of *Leptospira interrogans* serovar *Hardjo* in the Malaysian environment. J. Anim. Vet. Adv *3 (3)*, 123-129.

Khalil, H., Ecke, F., Evander, M., Bucht, G. and Hörnfeldt, B. (2019). Population Dynamics of Bank Voles Predicts Human Puumala Hantavirus Risk. Ecohealth *16 (3)*, 545-557, doi: 10.1007/s10393-019-01424-4.

Khalil, H., Ecke, F., Evander, M., Magnusson, M. and Hornfeldt, B. (2016). Declining ecosystem health and the dilution effect. Sci Rep *6*, 31314, doi: 10.1038/srep31314.

Khanakah, G., Kocianova, E., Vyrostekova, V., Rehacek, J., Kundi, M. and Stanek, G. (2006). Seasonal variations in detecting *Borrelia burgdorferi* sensu lato in rodents from north eastern Austria. Wien Klin Wochenschr *118 (23-24)*, 754-758, doi: 10.1007/s00508-006-0730-y.

Kim, C. M., Yi, Y. H., Yu, D. H., Lee, M. J., Cho, M. R., Desai, A. R., Shringi, S., Klein, T. A., Kim, H. C., Song, J. W., Baek, L. J., Chong, S. T., O'Guinn M, L., Lee, J. S., Lee, I. Y., Park, J. H., Foley, J. and Chae, J. S. (2006). Tick-borne rickettsial pathogens in ticks and small mammals in Korea. Appl Environ Microbiol *72 (9)*, 5766-5776, doi: 10.1128/aem.00431-06.

Kislichkina, A. A., Bogun, A. G., Kadnikova, L. A., Maiskaya, N. V., Solomentsev, V. I., Platonov, M. E., Dentovskaya, S. V. and Anisimov, A. P. (2017). Eight Whole-Genome Assemblies of *Yersinia pestis* subsp. *microtus* bv. caucasica Isolated from the Common Vole (*Microtus arvalis*) Plague Focus in Dagestan, Russia. Genome Announc. *5 (34)*, doi: 10.1128/genomeA.00847-17.

Klempa, B., Meisel, H., Rath, S., Bartel, J., Ulrich, R. and Krüger, D. H. (2003a). Occurrence of renal and pulmonary syndrome in a region of northeast Germany where Tula hantavirus circulates. J Clin Microbiol *41 (10)*, 4894-4897, doi: 10.1128/JCM.41.10.4894-4897.2003.

Klempa, B., Schmidt, H. A., Ulrich, R., Kaluz, S., Labuda, M., Meisel, H., Hjelle, B. and Krüger, D. H. (2003b). Genetic interaction between distinct Dobrava hantavirus subtypes in *Apodemus agrarius* and *A. flavicollis* in nature. J Virol *77 (1)*, 804-809, doi: 10.1128/jvi.77.1.804-809.2003.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Denys, C., Koivogui, L., ter Meulen, J. and Krüger, D. H. (2006). Hantavirus in African wood mouse, Guinea. Emerg Infect Dis *12 (5)*, 838-840, doi: 10.3201/eid1205.051487.

Klempa, B., Avsic-Zupanc, T., Clement, J., Dzagurova, T. K., Henttonen, H., Heyman, P., Jakab, F., Kruger, D. H., Maes, P., Papa, A., Tkachenko, E. A., Ulrich, R. G., Vapalahti, O. and Vaheri, A. (2013a). Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: definition of genotypes and their characteristics. Arch Virol *158 (3)*, 521-529, doi: 10.1007/s00705-012-1514-5.

Klempa, B., Radosa, L. and Kruger, D. H. (2013b). The broad spectrum of hantaviruses and their hosts in Central Europe. Acta Virol *57 (2)*, 130-137.

Klempa, B., Tkachenko, E. A., Dzagurova, T. K., Yunicheva, Y. V., Morozov, V. G., Okulova, N. M., Slyusareva, G. P., Smirnov, A. and Kruger, D. H. (2008). Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. Emerg Infect Dis *14 (4)*, 617-625, doi: 10.3201/eid1404.071310.

Knap, N., Duh, D., Birtles, R., Trilar, T., Petrovec, M. and Avsic-Zupanc, T. (2007). Molecular detection of *Bartonella* species infecting rodents in Slovenia. FEMS Immunol Med Microbiol *50 (1)*, 45-50, doi: 10.1111/j.1574-695X.2007.00226.x.

Knowles, S. C. (2011). The effect of helminth co-infection on malaria in mice: a meta-analysis. Int J Parasitol *41 (10)*, 1041-1051, doi: 10.1016/j.ijpara.2011.05.009.

Ko, A. I., Goarant, C. and Picardeau, M. (2009). *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol *7 (10)*, 736-747, doi: 10.1038/nrmicro2208.

Ko, S., Kang, J. G., Kim, H. C., Klein, T. A., Choi, K. S., Song, J. W., Youn, H. Y. and Chae, J. S. (2016). Prevalence, Isolation and Molecular Characterization of *Bartonella* Species in Republic of Korea. Transbound Emerg Dis *63 (1)*, 56-67, doi: 10.1111/tbed.12217.

Korva, M., Duh, D., Puterle, A., Trilar, T. and Zupanc, T. A. (2009). First molecular evidence of Tula hantavirus in *Microtus* voles in Slovenia. Virus Res *144 (1-2)*, 318-322, doi: 10.1016/j.virusres.2009.04.021.

Korva, M., Knap, N., Rus, K. R., Fajs, L., Grubelnik, G., Bremec, M., Knapic, T., Trilar, T. and Zupanc, T. A. (2013). Phylogeographic Diversity of Pathogenic and Non-Pathogenic Hantaviruses in Slovenia. Viruses *5 (12)*, 3071-3087, doi: 10.3390/v5123071.

Kotlik, P., Deffontaine, V., Mascheretti, S., Zima, J., Michaux, J. R. and Searle, J. B. (2006). A northern glacial refugium for bank voles (*Clethrionomys glareolus*). Proc Natl Acad Sci USA *103 (40)*, 14860-14864, doi: 10.1073/pnas.0603237103.

Kraljik, J., Paziewska-Harris, A., Miklisová, D., Blaňarová, L., Mošanský, L., Bona, M. and Stanko, M. (2016). Genetic diversity of *Bartonella* genotypes found in the striped field mouse (*Apodemus agrarius*) in Central Europe. Parasitology *143 (11)*, 1437-1442, doi: 10.1017/s0031182016000962.

Krøjgaard, L. H., Villumsen, S., Markussen, M. D., Jensen, J. S., Leirs, H. and Heiberg, A. C. (2009). High prevalence of *Leptospira* spp. in sewer rats (*Rattus norvegicus*). Epidemiol Infect *137 (11)*, 1586-1592, doi: 10.1017/s0950268809002647.

Krüger, D. H., Schönrich, G. and Klempa, B. (2011). Human pathogenic hantaviruses and prevention of infection. Hum Vaccin 7 (6), 685-693, doi: 10.4161/hv.7.6.15197

Kruger, D. H., Figueiredo, L. T., Song, J. W. and Klempa, B. (2015a). Hantaviruses--globally emerging pathogens. J Clin Virol *64*, 128-136, doi: 10.1016/j.jcv.2014.08.033.

Kruger, D. H., Tkachenko, E. A., Morozov, V. G., Yunicheva, Y. V., Pilikova, O. M., Malkin, G., Ishmukhametov, A. A., Heinemann, P., Witkowski, P. T., Klempa, B. and Dzagurova, T. K. (2015b). Life-Threatening Sochi Virus Infections, Russia. Emerg Infect Dis *21 (12)*, 2204-2208, doi: 10.3201/eid2112.150891.

Krüger, D. H., Ulrich, R. and Lundkvist, Ä. (2001). Hantavirus infections and their prevention. Microbes Infect *3 (13)*, 1129-1144, doi: 10.1016/s1286-4579(01)01474-5.

Kryštufek, B., Vohralík, V., Zima, J. & Zagorodnyuk, I. (2016). *Microtus agrestis* (errata version published in 2017). URL: https://www.iucnredlist.org/species/13426/11511 2050 [as of 24.05.2016].

Kudesia, G., Christie, P., Walker, E., Pinkerton, I. and Lloyd, G. (1988). DUAL INFECTION WITH LEPTOSPIRA AND HANTAVIRUS. Lancet *1 (8599)*, 1397.

Kumar, K. V., Lall, C., Raj, R. V., Vedhagiri, K. and Vijayachari, P. (2015). Coexistence and survival of pathogenic leptospires by formation of biofilm with *Azospirillum*. FEMS Microbiol Ecol *91 (6)*, doi: 10.1093/femsec/fiv051.

Kurucz, K., Madai, M., Bali, D., Hederics, D., Horvath, G., Kemenesi, G. and Jakab, F. (2018). Parallel Survey of Two Widespread Renal Syndrome-Causing Zoonoses: *Leptospira* spp. and *Hantavirus* in Urban Environment, Hungary. Vector Borne Zoonotic Dis *18 (4)*, 200-205, doi: 10.1089/vbz.2017.2204.

Kurucz, K., Hederics, D., Bali, D., Kemenesi, G., Horvath, G. and Jakab, F. (2019). Hepatitis E virus in Common voles (*Microtus arvalis*) from an urban environment, Hungary: Discovery of a Cricetidae-specific genotype of Orthohepevirus C. Zoonoses Public Health *66 (2)*, 259-263, doi: 10.1111/zph.12543.

Laenen, L., Vergote, V., Kafetzopoulou, L. E., Wawina, T. B., Vassou, D., Cook, J. A., Hugot, J. P., Deboutte, W., Kang, H. J., Witkowski, P. T., Köppen-Rung, P., Krüger, D. H., Licková, M., Stang, A., Strešková, L., Szemeš, T., Markowski, J., Hejduk, J., Kafetzopoulos, D., Van Ranst, M., Yanagihara, R., Klempa, B. and Maes, P. (2018). A Novel Hantavirus of the European Mole, Bruges Virus, Is Involved in Frequent Nova Virus Coinfections. Genome Biol Evol *10 (1)*, 45-55, doi: 10.1093/gbe/evx268.

Laenen, L., Vergote, V., Calisher, C. H., Klempa, B., Klingström, J., Kuhn, J. H. and Maes, P. (2019a). *Hantaviridae*: current classification and future perspectives. Viruses *11 (9)*, doi: 10.3390/v11090788.

Laenen, L., Vergote, V., Vanmechelen, B., Tersago, K., Baele, G., Lemey, P., Leirs, H., Dellicour, S., Vrancken, B. and Maes, P. (2019b). Identifying the patterns and drivers of Puumala hantavirus enzootic dynamics using reservoir sampling. Virus Evol *5 (1)*, vez009, doi: 10.1093/ve/vez009.

Latinne, A., Navascués, M., Pavlenko, M., Kartavtseva, I., Ulrich, R. G., Tiouchichine, M.-L., Catteau, G., Sakka, H., Quéré, J.-P. and Chelomina, G. (2020). Phylogeography of the striped field mouse, *Apodemus agrarius* (Rodentia: Muridae), throughout its distribution range in the Palaearctic region. Mamm Biol *100 (1)*, 19-31, doi: 10.1007/s42991-019-00001-0.

Lee, H. W., Lee, P. W. and Johnson, K. M. (1978). Isolation of the etiologic agent of Korean hemorrhagic fever. J Infect Dis *137 (3)*, 298-308, doi: 10.1093/infdis/137.3.298

Lehmann, J. S., Matthias, M. A., Vinetz, J. M. and Fouts, D. E. (2014). Leptospiral pathogenomics. Pathogens *3 (2)*, 280-308, doi: 10.3390/pathogens3020280.

Leukers, A. and Jacob, J. (2013). Projekt Umweltverträgliche Nagetierbekämpfung in der Landwirtschaft; Aufgabenpaket 1 a) Schadnagerprofile. URL: https://feldmaus.julius-kuehn.de/dokumente/upload/ab022_leukers_u_jacob2013schadnagerprofile.pdf [as of 03.05.2020].

Levett, P. N. (2001). Leptospirosis. Clin Microbiol Rev *14 (2)*, 296-326, doi: 10.1128/cmr.14.2.296-326.2001.

Li, S., Ojcius, D. M., Liao, S., Li, L., Xue, F., Dong, H. and Yan, J. (2010). Replication or death: distinct fates of pathogenic Leptospira strain Lai within macrophages of human or mouse origin. Innate Immun *16 (2)*, 80-92, doi: 10.1177/1753425909105580.

Ling, J., Sironen, T., Voutilainen, L., Hepojoki, S., Niemimaa, J., Isoviita, V. M., Vaheri, A., Henttonen, H. and Vapalahti, O. (2014). Hantaviruses in Finnish soricomorphs: evidence for two distinct hantaviruses carried by *Sorex araneus* suggesting ancient host-switch. Infect Genet Evol *27*, 51-61, doi: 10.1016/j.meegid.2014.06.023.

Linzey, A. V., Shar, S., Lkhagvasuren, D., Juškaitis, R., Sheftel, B., Meinig, H., Amori, G. & Henttonen, H. (2016). *Microtus oeconomus* (errata version published in 2017). URL: https://www.iucnredlist.org/species/13451/115113894 [as of 06.01.2020].

Literak, I. (1995). [Occurrence of *Coxiella burnetti* antibodies in cattle, sheep and small terrestrial mammals in the western region of Bohemia] [in Czech]. Vet Med (Praha) *40 (3)*, 77-80.

Liu, Q., Sun, J., Lu, L., Fu, G., Ding, G., Song, X., Meng, F., Wu, H., Yang, T., Ren, Z., Chen, E., Lin, J., Lv, H. and Chai, C. (2010). DETECTION OF *BARTONELLA* SPECIES IN SMALL MAMMALS FROM ZHEJIANG PROVINCE, CHINA. J Wildl Dis *46 (1)*, 179-185, doi: 10.7589/0090-3558-46.1.179.

Liu, R., Ma, H., Shu, J., Zhang, Q., Han, M., Liu, Z., Jin, X., Zhang, F. and Wu, X. (2019). Vaccines and Therapeutics Against Hantaviruses. Front Microbiol *10*, 2989, doi: 10.3389/fmicb.2019.02989.

Löhmus, M., Janse, I., van de Goot, F. and van Rotterdam, B. J. (2013). Rodents as potential couriers for bioterrorism agents. Biosecur Bioterror *11 Suppl 1*, S247-257, doi: 10.1089/bsp.2012.0085.

Loos-Frank, B. (1980). The common vole,*Microtus arvalis* pall. as intermediate host of*Mesocestoides* (Cestoda) in Germany. Z Parasitenkd *63 (2)*, 129-136.

Luis, A. D., Hayman, D. T., O'Shea, T. J., Cryan, P. M., Gilbert, A. T., Pulliam, J. R., Mills, J. N., Timonin, M. E., Willis, C. K., Cunningham, A. A., Fooks, A. R., Rupprecht, C. E., Wood, J. L. and Webb, C. T. (2013). A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proc Biol Sci *280 (1756)*, 20122753, doi: 10.1098/rspb.2012.2753.

Luque-Larena, J. J., Mougeot, F., Roig, D. V., Lambin, X., Rodriguez-Pastor, R., Rodriguez-Valin, E., Anda, P. and Escudero, R. (2015). Tularemia Outbreaks and Common Vole (*Microtus arvalis*) Irruptive Population Dynamics in Northwestern Spain, 1997-2014. Vector Borne Zoonotic Dis *15 (9)*, 568-570, doi: 10.1089/vbz.2015.1770.

Maas, M., de Vries, A., van Roon, A., Takumi, K., van der Giessen, J. and Rockx, B. (2017). High Prevalence of Tula Hantavirus in Common Voles in The Netherlands. Vector Borne Zoonotic Dis, doi: 10.1089/vbz.2016.1995.

Mada, P. K., Zulfiqar, H. and Joel Chandranesan, A. S. (2020). Bartonellosis. In: StatPearls, StatPearls Publishing LLC., Treasure Island (FL).

Maes, P., Alkhovsky, S. V., Bào, Y., Beer, M., Birkhead, M., Briese, T., Buchmeier, M. J., Calisher, C. H., Charrel, R. N. and Choi, I. R. (2018). Taxonomy of the family *Arenaviridae* and the order *Bunyavirales*: update 2018. Arch Virol *163 (8)*, 2295-2310, doi: 10.1007/s00705-018-3843-5.

Markotic, A., Kuzman, I., Babic, K., Gagro, A., Nichol, S., Ksiazek, T. G., Rabatic, S. and Dekaris, D. (2002a). Double trouble: hemorrhagic fever with renal syndrome and leptospirosis. Scand J Infect Dis *34 (3)*, 221-224.

Markotic, A., Nichol, S. T., Kuzman, I., Sanchez, A. J., Ksiazek, T. G., Gagro, A., Rabatic, S., Zgorelec, R., Avsic-Zupanc, T., Beus, I. and Dekaris, D. (2002b). Characteristics of Puumala and Dobrava infections in Croatia. J Med Virol *66 (4)*, 542-551, doi: 10.1002/jmv.2179.

Marquez, A., Djelouadji, Z., Lattard, V. and Kodjo, A. (2017). Overview of laboratory methods to diagnose Leptospirosis and to identify and to type leptospires. Int Microbiol *20 (4)*, 184-193, doi: 10.2436/20.1501.01.302.

Mason, M. R., Encina, C., Sreevatsan, S. and Munoz-Zanzi, C. (2016). Distribution and Diversity of Pathogenic *Leptospira* Species in Peri-domestic Surface Waters from South Central Chile. PLoS Negl Trop Dis *10 (8)*, e0004895, doi: 10.1371/journal.pntd.0004895.

Masuzawa, T., Pan, M. J., Kadosaka, T., Kudeken, M., Takada, N., Yano, Y., Imai, Y. and Yanagihara, Y. (2000). Characterization and identification of *Borrelia* isolates as *Borrelia valaisiana* in Taiwan and Kinmen Islands. Microbiol Immunol *44 (12)*, 1003-1009, doi: 10.1111/j.1348-0421.2000.tb02596.x.

Masuzawa, T., Takada, N., Kudeken, M., Fukui, T., Yano, Y., Ishiguro, F., Kawamura, Y., Imai, Y. and Ezaki, T. (2001). *Borrelia sinica* sp. nov., a lyme disease-related *Borrelia* species isolated in China. Int J Syst Evol Microbiol *51 (Pt 5)*, 1817-1824, doi: 10.1099/00207713-51-5-1817.

Matei, I. A., D'Amico, G., Ionica, A. M., Kalmar, Z., Corduneanu, A., Sandor, A. D., Fit, N., Bogdan, L., Gherman, C. M. and Mihalca, A. D. (2018). New records for *Anaplasma phagocytophilum* infection in small mammal species. Parasit Vectors *11 (1)*, 193, doi: 10.1186/s13071-018-2791-y.

Mayer-Scholl, A., Draeger, A., Luge, E., Ulrich, R. and Nockler, K. (2011). Comparison of two PCR systems for the rapid detection of Leptospira spp. from kidney tissue. Curr Microbiol *62 (4)*, 1104-1106, doi: 10.1007/s00284-010-9829-5.

Mayer-Scholl, A., Hammerl, J. A., Schmidt, S., Ulrich, R. G., Pfeffer, M., Woll, D., Scholz, H. C., Thomas, A. and Nockler, K. (2014). *Leptospira* spp. in rodents and shrews in Germany. Int J Environ Res Public Health *11 (8)*, 7562-7574, doi: 10.3390/ijerph 110807562.

Mediannikov, O., Ivanov, L., Zdanovskaya, N., Vysochina, N., Fournier, P. E., Tarasevich, I. and Raoult, D. (2005). Molecular screening of *Bartonella* species in rodents from the Russian Far East. Ann N Y Acad Sci *1063*, 308-311, doi: 10.1196/annals.1355.049.

Meerburg, B. G., Singleton, G. R. and Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. Critical reviews in microbiology *35 (3)*, 221-270, doi: doi:10.1080/10408410902989837.

Mertens, M., Essbauer, S. S., Rang, A., Schröder, J., Splettstoesser, W. D., Kretzschmar, C., Krüger, D. H., Groschup, M. H., Matz-Rensing, K. and Ulrich, R. G. (2011a). Non-

human primates in outdoor enclosures: risk for infection with rodent-borne hantaviruses. Vet Microbiol *147 (3-4)*, 420-425, doi: 10.1016/j.vetmic.2010.07.018.

Mertens, M., Hofmann, J., Petraityte-Burneikiene, R., Ziller, M., Sasnauskas, K., Friedrich, R., Niederstrasser, O., Krüger, D. H., Groschup, M. H., Petri, E., Werdermann, S. and Ulrich, R. G. (2011b). Seroprevalence study in forestry workers of a non-endemic region in eastern Germany reveals infections by Tula and Dobrava-Belgrade hantaviruses. Med Microbiol Immunol *200 (4)*, 263-268, doi: 10.1007/s00430-011-0203-4.

Mgode, G. F., Mhamphi, G. G., Katkweba, A. S. and Thomas, M. (2014). Leptospira infections in freshwater fish in Morogoro Tanzania: a hidden public health threat. Tanzan J Health Res *16 (2)*, 112-117.

Michalski, A., Niemcewicz, M., Bielawska-Drozd, A., Nowakowska, A., Gawel, J., Pitucha, G., Joniec, J., Zielonka, K., Marciniak-Niemcewicz, A. and Kocik, J. (2014). Surveillance of hantaviruses in Poland: a study of animal reservoirs and human hantavirus disease in Subcarpathia. Vector Borne Zoonotic Dis *14 (7)*, 514-522, doi: 10.1089/vbz.2013.1468.

Millan, J., Cevidanes, A., Chirife, A. D., Candela, M. G. and Leon-Vizcaino, L. (2018). Risk factors of *Leptospira* infection in Mediterranean periurban micromammals. Zoonoses Public Health *65 (1)*, e79-e85, doi: 10.1111/zph.12411.

Miller, R. I., Ross, S. P., Sullivan, N. D. and Perkins, N. R. (2007). Clinical and epidemiological features of canine leptospirosis in North Queensland. Aust Vet J *85 (1-2)*, 13-19, doi: 10.1111/j.1751-0813.2006.00089.x.

Mix, A. C., Bard, E. and Schneider, R. (2001). Environmental processes of the ice age: land, oceans, glaciers (EPILOG). Quat Sci Rev *20 (4)*, 627-657, doi: 10.1016/S0277-3791(00)00145-1.

Mitchell, C. J., Lvov, S. D., Savage, H. M., Calisher, C. H., Smith, G. C., Lvov, D. K. and Gubler, D. J. (1993). Vector and host relationships of California serogroup viruses in western Siberia. Am J Trop Med Hyg 49 (1), 53-62, doi: 10.4269/ajtmh.1993.49.53. .

Monahan, A. M., Callanan, J. J. and Nally, J. E. (2008). Proteomic analysis of *Leptospira interrogans* shed in urine of chronically infected hosts. Infect Immun *76 (11)*, 4952-4958, doi: 10.1128/iai.00511-08.

Monahan, A. M., Callanan, J. J. and Nally, J. E. (2009). Review paper: Host-pathogen interactions in the kidney during chronic leptospirosis. Vet Pathol *46 (5)*, 792-799, doi: 10.1354/vp.08-VP-0265-N-REV.

Monchatre-Leroy, E., Crespin, L., Boué, F., Marianneau, P., Calavas, D. and Hénaux, V. (2017). Spatial and Temporal Epidemiology of Nephropathia Epidemica Incidence and Hantavirus Seroprevalence in Rodent Hosts: Identification of the Main Environmental Factors in Europe. Transbound Emerg Dis *64 (4)*, 1210-1228, doi: 10.1111/tbed.12494.

Morand, S., Blasdell, K., Bordes, F., Buchy, P., Carcy, B., Chaisiri, K., Chaval, Y., Claude, J., Cosson, J. F., Desquesnes, M., Jittapalapong, S., Jiyipong, T., Karnchanabanthoen, A., Pornpan, P., Rolain, J. M. and Tran, A. (2019). Changing landscapes of Southeast Asia

and rodent-borne diseases: decreased diversity but increased transmission risks. Ecol Appl *29 (4)*, e01886, doi: 10.1002/eap.1886.

Morgan, J., Bornstein, S. L., Karpati, A. M., Bruce, M., Bolin, C. A., Austin, C. C., Woods, C. W., Lingappa, J., Langkop, C., Davis, B., Graham, D. R., Proctor, M., Ashford, D. A., Bajani, M., Bragg, S. L., Shutt, K., Perkins, B. A. and Tappero, J. W. (2002). Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998. Clin Infect Dis *34 (12)*, 1593-1599, doi: 10.1086/340615.

Muyangwa, M., Martynova, E. V., Khaiboullina, S. F., Morzunov, S. P. and Rizvanov, A. A. (2015). Hantaviral Proteins: Structure, Functions, and Role in Hantavirus Infection. Front Microbiol *6*, 1326, doi: 10.3389/fmicb.2015.01326.

Nainys, J., Timinskas, A., Schneider, J., Ulrich, R. G. and Gedvilaite, A. (2015). Identification of Two Novel Members of the Tentative Genus Wukipolyomavirus in Wild Rodents. PLoS One *10 (10)*, e0140916, doi: 10.1371/journal.pone.0140916.

Nally, J. E., Chow, E., Fishbein, M. C., Blanco, D. R. and Lovett, M. A. (2005). Changes in lipopolysaccharide O antigen distinguish acute versus chronic Leptospira interrogans infections. Infect Immun *73 (6)*, 3251-3260, doi: 10.1128/iai.73.6.3251-3260.2005.

Nemes, Z. and Péterfi, Z. (2000). [Simultaneous leptospirosis and hantavirus infection in the same patient] [in Hungarian]. Orv Hetil *141 (10)*, 499-502.

Nemeth, V., Madai, M., Maraczi, A., Berczi, B., Horvath, G., Oldal, M., Kisfali, P., Banyai, K. and Jakab, F. (2011). Detection of Dobrava-Belgrade hantavirus using recombinant-nucleocapsid-based enzyme-linked immunosorbent assay and SYBR Green-based real-time reverse transcriptase-polymerase chain reaction. Arch Virol *156 (9)*, 1655-1660, doi: 10.1007/s00705-011-1013-0.

Nemirov, K., Henttonen, H., Vaheri, A. and Plyusnin, A. (2002). Phylogenetic evidence for host switching in the evolution of hantaviruses carried by *Apodemus* mice. Virus Res *90 (1-2)*, 207-215, doi: 10.1016/s0168-1702(02)00179-x.

Nemirov, K., Vapalahti, O., Papa, A., Plyusnina, A., Lundkvist, Ä., Antoniadis, A., Vaheri, A. and Plyusnin, A. (2003). Genetic characterization of new Dobrava hantavirus isolate from Greece. J Med Virol *69 (3)*, 408-416, doi: 10.1002/jmv.10304.

Nemirov, K., Andersen, H. K., Leirs, H., Henttonen, H., Vaheri, A., Lundkvist, Ä. and Plyusnin, A. (2004). Saaremaa hantavirus in Denmark. J Clin Virol *30 (3)*, 254-257, doi: 10.1016/j.jcv.2003.12.009.

Niklasson, B., Hornfeldt, B., Lundkvist, Ä., Bjorsten, S. and Leduc, J. (1995). Temporal dynamics of Puumala virus antibody prevalence in voles and of nephropathia epidemica incidence in humans. Am J Trop Med Hyg *53 (2)*, 134-140.

Novakova, D., Pantucek, R., Hubalek, Z., Falsen, E., Busse, H. J., Schumann, P. and Sedlacek, I. (2010). *Staphylococcus microti* sp. nov., isolated from the common vole (*Microtus arvalis*). Int J Syst Evol Microbiol *60 (Pt 3)*, 566-573, doi: 10.1099/ijs.0.011429-0.

Obiegala, A., Woll, D., Karnath, C., Silaghi, C., Schex, S., Essbauer, S. and Pfeffer, M. (2016). Prevalence and Genotype Allocation of Pathogenic *Leptospira* Species in Small Mammals from Various Habitat Types in Germany. PLoS Negl Trop Dis *10 (3)*, e0004501, doi: 10.1371/journal.pntd.0004501.

Obiegala, A., Albrecht, C., Dafalla, M., Drewes, S., Oltersdorf, C., Turni, H., Imholt, C., Jacob, J., Wagner-Wiening, C., Ulrich, R. G. and Pfeffer, M. (2017a). *Leptospira* spp. in Small Mammals from Areas with Low and High Human Hantavirus Incidences in South-West Germany. Vector Borne Zoonotic Dis *17 (5)*, 312-318, doi: 10.1089/vbz.2016.2036.

Obiegala, A., Krol, N., Oltersdorf, C., Nader, J. and Pfeffer, M. (2017b). The enzootic life-cycle of *Borrelia burgdorferi* (sensu lato) and tick-borne rickettsiae: an epidemiological study on wild-living small mammals and their ticks from Saxony, Germany. Parasit Vectors *10 (1)*, 115, doi: 10.1186/s13071-017-2053-4.

Obiegala, A., Jeske, K., Augustin, M., Krol, N., Fischer, S., Mertens-Scholz, K., Imholt, C., Suchomel, J., Heroldova, M., Tomaso, H., Ulrich, R. G. and Pfeffer, M. (2019). Highly prevalent bartonellae and other vector-borne pathogens in small mammal species from the Czech Republic and Germany. Parasit Vectors *12 (1)*, 332, doi: 10.1186/s13071-019-3576-7.

Oktem, I. M., Uyar, Y., Dincer, E., Gozalan, A., Schlegel, M., Babur, C., Celebi, B., Sozen, M., Karatas, A., Ozkazanc, N. K., Matur, F., Korukluoglu, G., Ulrich, R. G., Ertek, M. and Ozkul, A. (2014). Dobrava-Belgrade virus in *Apodemus flavicollis* and *A. uralensis* mice, Turkey. Emerg Infect Dis *20 (1)*, 121-125, doi: 10.3201/eid2001.121024.

Olsson, G. E., Leirs, H. and Henttonen, H. (2010). Hantaviruses and their hosts in Europe: reservoirs here and there, but not everywhere? Vector Borne Zoonotic Dis *10 (6)*, 549-561, doi: 10.1089/vbz.2009.0138.

Padula, P. J., Edelstein, A., Miguel, S. D., Lopez, N. M., Rossi, C. M. and Rabinovich, R. D. (1998). Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology *241 (2)*, 323-330, doi: 10.1006/viro.1997.8976.

Padula, P., Figueroa, R., Navarrete, M., Pizarro, E., Cadiz, R., Bellomo, C., Jofre, C., Zaror, L., Rodriguez, E. and Murúa, R. (2004). Transmission study of Andes hantavirus infection in wild sigmodontine rodents. J Virol *78 (21)*, 11972-11979, doi: 10.1128/jvi.78.21.11972-11979.2004.

Papa, A., Bojovic, B. and Antoniadis, A. (2006). Hantaviruses in Serbia and Montenegro. Emerg Infect Dis *12 (6)*, 1015-1018, doi: 10.3201/eid1206.051564.

Papa, A. and Christova, I. (2011). Genetic Detection of Dobrava/Belgrade Virus, Bulgaria. Emerg Infect Dis *17 (2)*, 308-309, doi: 10.3201/eid1702.101275.

Pardiñas, U., Kryštufek, B., Bradley, R., Ordóñez-Garza, N., Cook, J., Shenbrot, G., Haslauer, R., León-Paniagua, L. and Brito, J. (2017). Handbook of the Mammals of the World: Rodents II, Lynx Edicions in association with Conservation International and IUCN, Barcelona.

Park, J. W., Chung, J. K., Kim, S. H., Cho, S. J., Ha, Y. D., Jung, S. H., Park, H. J., Song, H. J., Lee, J. Y., Kim, D. M., Pyus, J., Ha, D. R., Kim, E. S. and Lee, J. I. (2016). Seroepidemiological Survey of Zoonotic Diseases in Small Mammals with PCR Detection of *Orientia tsutsugamushi* in Chiggers, Gwangju, Korea. Korean J Parasitol *54 (3)*, 307-313, doi: 10.3347/kjp.2016.54.3.307.

Park, K. H., Chang, W. H. and Schwan, T. G. (1993). Identification and characterization of Lyme disease spirochetes, *Borrelia burgdorferi* sensu lato, isolated in Korea. J Clin Microbiol *31 (7)*, 1831-1837, doi: 10.1128/jcm.31.7.1831-1837.1993.

Pathak, A. K., Pelensky, C., Boag, B. and Cattadori, I. M. (2012). Immuno-epidemiology of chronic bacterial and helminth co-infections: observations from the field and evidence from the laboratory. Int J Parasitol *42 (7)*, 647-655, doi: 10.1016/j.ijpara.2012.04.011.

Pawelczyk, A., Bajer, A., Behnke, J. M., Gilbert, F. S. and Sinski, E. (2004). Factors affecting the component community structure of haemoparasites in common voles (*Microtus arvalis*) from the Mazury Lake District region of Poland. Parasitol Res *92 (4)*, 270-284, doi: 10.1007/s00436-003-1040-1.

Paziewska, A., Harris, P. D., Zwolinska, L., Bajer, A. and Sinski, E. (2011). Recombination within and between species of the alpha proteobacterium *Bartonella* infecting rodents. Microb Ecol *61 (1)*, 134-145, doi: 10.1007/s00248-010-9735-1.

Pejcoch, M., Heroldova, M., Zejda, J., Treml, F. and Kriz, B. (2003). [Detection of hantavirus antigen in rodents in the Czech Republic] [in Czech]. Epidemiol Mikrobiol Imunol *52 (1)*, 18-24.

Perez, J., Brescia, F., Becam, J., Mauron, C. and Goarant, C. (2011). Rodent abundance dynamics and leptospirosis carriage in an area of hyper-endemicity in New Caledonia. PLoS Negl Trop Dis *5 (10)*, e1361, doi: 10.1371/journal.pntd.0001361.

Pinter, R., Madai, M., Horvath, G., Nemeth, V., Oldal, M., Kemenesi, G., Dallos, B., Banyai, K. and Jakab, F. (2014). Molecular detection and phylogenetic analysis of tick-borne encephalitis virus in rodents captured in the transdanubian region of Hungary. Vector Borne Zoonotic Dis *14 (8)*, 621-624, doi: 10.1089/vbz.2013.1479.

Phillips, B. L., Kelehear, C., Pizzatto, L., Brown, G. P., Barton, D. and Shine, R. (2010). Parasites and pathogens lag behind their host during periods of host range advance. Ecology *91 (3)*, 872-881, doi: 10.1890/09-0530.1.

Plourde, B. T., Burgess, T. L., Eskew, E. A., Roth, T. M., Stephenson, N. and Foley, J. E. (2017). Are disease reservoirs special? Taxonomic and life history characteristics. PLoS One *12 (7)*, e0180716, doi: 10.1371/journal.pone.0180716.

Plyusnin, A., Vapalahti, O., Lankinen, H., Lehväslaiho, H., Apekina, N., Myasnikov, Y., Kallio-Kokko, H., Henttonen, H., Lundkvist, Ä. and Brummer-Korvenkontio, M. (1994). Tula virus: a newly detected hantavirus carried by European common voles. J Virol *68 (12)*, 7833-7839.

Plyusnin, A., Vapalahti, O., Vasilenko, V., Henttonen, H. and Vaheri, A. (1997). Dobrava hantavirus in Estonia: does the virus exist throughout Europe? Lancet *349 (9062)*, 1369-1370, doi: 10.1016/s0140-6736(05)63206-0.

Plyusnin, A., Nemirov, K., Apekina, N., Plyusnina, A., Lundkvist, Ä. and Vaheri, A. (1999). Dobrava hantavirus in Russia. Lancet *353 (9148)*, 207, doi: 10.1016/s0140-6736(05)77216-0.

Plyusnin, A. (2002). Genetics of hantaviruses: implications to taxonomy. Arch Virol *147 (4)*, 665-682, doi: 10.1007/s007050200017.

Plyusnina, A., Laakkonen, J., Niemimaa, J., Henttonen, H. and Plyusnin, A. (2008a). New Genetic Lineage of Tula Hantavirus in *Microtus arvalis obscurus* in Eastern Kazakhstan. Open Virol J. *2*, 32-36, doi: 10.2174/1874357900802010032.

Plyusnina, A., Laakkonen, J., Niemimaa, J., Nemirov, K., Muruyeva, G., Pohodiev, B., Lundkvist, Ä., Vaheri, A., Henttonen, H., Vapalahti, O. and Plyusnin, A. (2008b). Genetic analysis of hantaviruses carried by *Myodes* and *Microtus* rodents in Buryatia. Virol J *5*, 4, doi: 10.1186/1743-422x-5-4.

Plyusnina, A., Ferenczi, E., Racz, G. R., Nemirov, K., Lundkvist, Ä., Vaheri, A., Vapalahti, O. and Plyusnin, A. (2009). Co-circulation of three pathogenic hantaviruses: Puumala, Dobrava, and Saaremaa in Hungary. J Med Virol *81 (12)*, 2045-2052, doi: 10.1002/jmv.21635.

Plyusnina, A., Krajinovic, L. C., Margaletic, J., Niemimaa, J., Nemirov, K., Lundkvist, Ä., Markotic, A., Miletic-Medved, M., Avšič-Županc, T., Henttonen, H. and Plyusnin, A. (2011). Genetic evidence for the presence of two distinct hantaviruses associated with *Apodemus* mice in Croatia and analysis of local strains. J Med Virol *83 (1)*, 108-114, doi: 10.1002/jmv.21929.

Polat, C., Ergunay, K., Irmak, S., Erdin, M., Brinkmann, A., Cetintas, O., Cogal, M., Sozen, M., Matur, F., Nitsche, A. and Oktem, I. M. A. (2018a). A novel genetic lineage of Tula orthohantavirus in Altai voles (*Microtus obscurus*) from Turkey. Infect Genet Evol *67*, 150-158, doi: 10.1016/j.meegid.2018.11.015.

Polat, C., Sironen, T., Plyusnina, A., Karatas, A., Sozen, M., Matur, F., Vapalahti, O., Oktem, I. M. A. and Plyusnin, A. (2018b). Dobrava hantavirus variants found in *Apodemus flavicollis* mice in Kirklareli Province, Turkey. J Med Virol *90 (5)*, 810-818, doi: 10.1002/jmv.25036.

Popp, L. (1960). The epidemiology of field fever in the foothills of Lower Saxony. Int. J. Hyg. Environ. Health *144*, 345.

Portillo, A., Santibáñez, P., Palomar, A. M., Santibáñez, S. and Oteo, J. A. (2018). '*Candidatus* Neoehrlichia mikurensis' in Europe. New Microbes New Infect *22*, 30-36, doi: 10.1016/j.nmni.2017.12.011.

Pounder, K. C., Begon, M., Sironen, T., Henttonen, H., Watts, P. C., Voutilainen, L., Vapalahti, O., Klempa, B., Fooks, A. R. and McElhinney, L. M. (2013). Novel Hantavirus in Wildlife, United Kingdom. Emerg Infect Dis *19 (4)*, 673-675, doi: 10.3201/eid1904.121057.

Power, A. G. and Mitchell, C. E. (2004). Pathogen spillover in disease epidemics. Am Nat *164 Suppl 5*, S79-89, doi: 10.1086/424610.

Prkno, A., Hoffmann, D., Goerigk, D., Kaiser, M., van Maanen, A. C. F., Jeske, K., Jenckel, M., Pfaff, F., Vahlenkamp, T. W., Beer, M., Ulrich, R. G., Starke, A. and Pfeffer, M. (2017). Epidemiological Investigations of Four Cowpox Virus Outbreaks in Alpaca Herds, Germany. Viruses *9 (11)*, doi: 10.3390/v9110344.

Qin, X. R., Liu, J. W., Yu, H. and Yu, X. J. (2019). *Bartonella* Species Detected in Rodents from Eastern China. Vector Borne Zoonotic Dis *19 (11)*, 810-814, doi: 10.1089/vbz.2018.2410.

Radosa, L., Schlegel, M., Gebauer, P., Ansorge, H., Heroldová, M., Jánová, E., Stanko, M., Mošanský, L., Fričová, J., Pejčoch, M., Suchomel, J., Purchart, L., Groschup, M. H., Krüger, D. H., Ulrich, R. G. and Klempa, B. (2013). Detection of shrew-borne hantavirus in Eurasian pygmy shrew (*Sorex minutus*) in Central Europe. Infect Genet Evol *19*, 403-410, doi: 10.1016/j.meegid.2013.04.008.

Rajapakse, S., Rodrigo, C., Balaji, K. and Fernando, S. D. (2015). Atypical manifestations of leptospirosis. Trans R Soc Trop Med Hyg *109 (5)*, 294-302.

Rasche, F. M., Schmidt, S., Kretzschmar, C., Mertens, M., Thiel, J., Groschup, M. H., Schlegel, M., Mayer, C., Lindner, T. H., Schiekofer, S. and Ulrich, R. G. (2015). Autochthonous Dobrava-Belgrade virus infection in Eastern Germany. Clin Nephrol *83 (2)*, 111-116, doi: 10.5414/cn108192.

Ratet, G., Veyrier, F. J., Fanton d'Andon, M., Kammerscheit, X., Nicola, M. A., Picardeau, M., Boneca, I. G. and Werts, C. (2014). Live Imaging of Bioluminescent *Leptospira interrogans* in Mice Reveals Renal Colonization as a Stealth Escape from the Blood Defenses and Antibiotics. PLoS Negl Trop Dis *8 (12)*, e3359, doi: 10.1371/journal.pntd.0003359.

Razzauti, M., Plyusnina, A., Niemimaa, J., Henttonen, H. and Plyusnin, A. (2012). Co-circulation of two Puumala hantavirus lineages in Latvia: a Russian lineage described previously and a novel Latvian lineage. J Med Virol *84 (2)*, 314-318, doi: 10.1002/jmv.22263.

Reil, D., Imholt, C., Eccard, J. A. and Jacob, J. (2015). Beech Fructification and Bank Vole Population Dynamics--Combined Analyses of Promoters of Human Puumala Virus Infections in Germany. PLoS One *10 (7)*, e0134124, doi: 10.1371/journal.pone.0134124.

Reil, D., Rosenfeld, U. M., Imholt, C., Schmidt, S., Ulrich, R. G., Eccard, J. A. and Jacob, J. (2017). Puumala hantavirus infections in bank vole populations: host and virus dynamics in Central Europe. BMC Ecol *17 (1)*, 9, doi: 10.1186/s12898-017-0118-z.

Reis, R. B., Ribeiro, G. S., Felzemburgh, R. D., Santana, F. S., Mohr, S., Melendez, A. X., Queiroz, A., Santos, A. C., Ravines, R. R., Tassinari, W. S., Carvalho, M. S., Reis, M. G. and Ko, A. I. (2008). Impact of environment and social gradient on *Leptospira* infection in urban slums. PLoS Negl Trop Dis *2 (4)*, e228, doi: 10.1371/journal.pntd.0000228.

Ren, Y. R., Golding, A., Sorbello, A., Ji, P., Chen, J., Bhawana, S., Witzmann, K., Arya, V., Reynolds, K. S., Choi, S. Y., Nikolov, N. and Sahajwalla, C. (2020). A Comprehensive Updated Review on SARS-CoV-2 and COVID-19. J Clin Pharmacol, doi: 10.1002/jcph.1673.

Reusken, C., de Vries, A., Adema, J., Vos, W., van der Giessen, J., Bekker, D. and Heyman, P. (2008). First genetic detection of Tula hantavirus in wild rodents in the Netherlands. J Infect *57 (6)*, 500-503, doi: 10.1016/j.jinf.2008.09.032.

Reynes, J. M., Carli, D., Boukezia, N., Debruyne, M. and Herti, S. (2015). Tula hantavirus infection in a hospitalised patient, France, June 2015. Euro Surveill *20 (50)*, doi: 10.2807/1560-7917.es.2015.20.50.30095.

Richter, D., Klug, B., Spielman, A. and Matuschka, F. R. (2004). Adaptation of diverse lyme disease spirochetes in a natural rodent reservoir host. Infect Immun *72 (4)*, 2442-2444, doi: 10.1128/iai.72.4.2442-2444.2004.

Riebold, D., Russow, K., Schlegel, M., Wollny, T., Thiel, J., Freise, J., Hüppop, O., Eccard, J. A., Plenge-Bönig, A., Loebermann, M., Ulrich, R. G., Klammt, S., Mettenleiter, T. C. and Reisinger, E. C. (2020). Occurrence of Gastrointestinal Parasites in Small Mammals from Germany. Vector Borne Zoonotic Dis *20 (2)*, 125-133, doi: 10.1089/vbz.2019.2457.

Ristow, P., Bourhy, P., Kerneis, S., Schmitt, C., Prevost, M. C., Lilenbaum, W. and Picardeau, M. (2008). Biofilm formation by saprophytic and pathogenic leptospires. Microbiology *154 (Pt 5)*, 1309-1317, doi: 10.1099/mic.0.2007/014746-0.

Rivera, P. C., González-Ittig, R. E. and Gardenal, C. N. (2015). Preferential host switching and its relation with Hantavirus diversification in South America. J Gen Virol *96 (9)*, 2531-2542, doi: 10.1099/vir.0.000210.

Rizzoli, A., Tagliapietra, V., Rosa, R., Hauffe, H. C., Marini, G., Voutilainen, L., Sironen, T., Rossi, C., Arnoldi, D. and Henttonen, H. (2015). Recent increase in prevalence of antibodies to Dobrava-Belgrade virus (DOBV) in yellow-necked mice in Northern Italy. Epidemiol Infect *143 (10)*, 2241-2244, doi: 10.1017/s0950268814003525.

Rodriguez-Pastor, R., Escudero, R., Vidal, D., Mougeot, F., Arroyo, B., Lambin, X., Vila-Coro, A. M., Rodriguez-Moreno, I., Anda, P. and Luque-Larena, J. J. (2017). Density-Dependent Prevalence of *Francisella tularensis* in Fluctuating Vole Populations, Northwestern Spain. Emerg Infect Dis *23 (8)*, 1377-1379, doi: 10.3201/eid2308.161194.

Rodriguez-Pastor, R., Escudero, R., Lambin, X., Vidal, M. D., Gil, H., Jado, I., Rodriguez-Vargas, M., Luque-Larena, J. J. and Mougeot, F. (2018). Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. Parasitology, 1-10, doi: 10.1017/s0031182018001543.

Rojas, P., Monahan, A. M., Schuller, S., Miller, I. S., Markey, B. K. and Nally, J. E. (2010). Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. Eur J Clin Microbiol Infect Dis *29 (10)*, 1305-1309, doi: 10.1007/s10096-010-0991-2.

Rosenfeld, U. M., Drewes, S., Ali, H. S., Sadowska, E. T., Mikowska, M., Heckel, G., Koteja, P. and Ulrich, R. G. (2017). A highly divergent Puumala virus lineage in southern Poland. Arch Virol *162 (5)*, 1177-1185, doi: 10.1007/s00705-016-3200-5.

Ryll, R., Heckel, G., Corman, V. M., Drexler, J. F. and Ulrich, R. G. (2019). Genomic and spatial variability of a European common vole hepevirus. Arch Virol *164 (11)*, 2671-2682, doi: 10.1007/s00705-019-04347-1.

Sackl, P., Tiefenbach, M., Tajmel, J. and Spitzenberger, F. (2007). Further spread of the striped field mouse *Apodemus agrarius* (PALLAS, 1771) in Austria (Mammalia). Joannea Zool. *(9)*, 5-13.

Sachsenröder, J., Braun, A., Machnowska, P., Ng, T. F. F., Deng, X., Guenther, S., Bernstein, S., Ulrich, R. G., Delwart, E. and Johne, R. (2014). Metagenomic identification of novel enteric viruses in urban wild rats and genome characterization of a group A rotavirus. J Gen Virol *95 (Pt 12)*, 2734-2747, doi: 10.1099/vir.0.070029-0.

Sauvage, F., Penalba, C., Vuillaume, P., Boue, F., Coudrier, D., Pontier, D. and Artois, M. (2002). Puumala hantavirus infection in humans and in the reservoir host, Ardennes region, France. Emerg Infect Dis *8 (12)*, 1509-1511, doi: 10.3201/eid0812.010518.

Saxenhofer, M., Schmidt, S., Ulrich, R. G. and Heckel, G. (2019). Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus. PLoS Biol *17 (2)*, e3000142, doi: 10.1371/journal.pbio.3000142.

Scharninghausen, J. J., Meyer, H., Pfeffer, M., Davis, D. S. and Honeycutt, R. L. (1999). Genetic evidence of Dobrava virus in *Apodemus agrarius* in Hungary. Emerg Infect Dis *5 (3)*, 468-470, doi: 10.3201/eid0503.990324.

Scharninghausen, J. J., Pfeffer, M., Meyer, H., Davis, D. S., Honeycutt, R. L. and Faulde, M. (2002). Genetic Evidence for Tula Virus in *Microtus arvalis* and *Microtus agrestis* Populations in Croatia. Vector Borne Zoonotic Dis *2 (1)*, 19-27, doi: 10.1089/153036602760260742.

Schlegel, M., Klempa, B., Auste, B., Bemmann, M., Schmidt-Chanasit, J., Buchner, T., Groschup, M. H., Meier, M., Balkema-Buschmann, A., Zoller, H., Krüger, D. H. and Ulrich, R. G. (2009). Dobrava-Belgrade Virus Spillover Infections, Germany. Emerg Infect Dis *15 (12)*, 2017-2020, doi: 10.3201/eid1512.090923.

Schlegel, M., Kindler, E., Essbauer, S. S., Wolf, R., Thiel, J., Groschup, M. H., Heckel, G., Oehme, R. M. and Ulrich, R. G. (2012). Tula virus infections in the Eurasian water vole in Central Europe. Vector Borne Zoonotic Dis *12 (6)*, 503-513, doi: 10.1089/vbz.2011.0784.

Schlegel, M., Jacob, J., Krüger, D. H., Rang, A. and Ulrich, R. G. (2014). Hantavirus emergence in rodents, insectivores and bats: what comes next? In: The role of animals in emerging viral diseases, ed. Johnson, N., Academic Press Amsterdamm, pp. 235-292.

Schlitter, D., van der Straeten, E., Amori, G., Hutterer, R., Kryštufek, B., Yigit, N. & Mitsain, G (2016). *Apodemus sylvaticus* (errata version published in 2017). URL: https://www.iucnredlist.org/species/1904/115059104 [as of 24.05.2020].

Schmaljohn, C. S. (1996). Molecular biology of hantaviruses. In: The Bunyaviridae, ed. Elliott, R. M., Springer, Boston, MA, pp. 63-90.

Schmidt-Chanasit, J., Essbauer, S., Petraityte, R., Yoshimatsu, K., Tackmann, K., Conraths, F. J., Sasnauskas, K., Arikawa, J., Thomas, A., Pfeffer, M., Scharninghausen, J. J., Splettstoesser, W., Wenk, M., Heckel, G. and Ulrich, R. G. (2010). Extensive host sharing of central European Tula virus. J Virol *84 (1)*, 459-474, doi: 10.1128/jvi.01226-09.

Schmidt, S., Essbauer, S. S., Mayer-Scholl, A., Poppert, S., Schmidt-Chanasit, J., Klempa, B., Henning, K., Schares, G., Groschup, M. H., Spitzenberger, F., Richter, D., Heckel, G. and Ulrich, R. G. (2014). Multiple infections of rodents with zoonotic pathogens in Austria. Vector Borne Zoonotic Dis *14 (7)*, 467-475, doi: 10.1089/vbz.2013.1504.

Schmidt, S., Saxenhofer, M., Drewes, S., Schlegel, M., Wanka, K. M., Frank, R., Klimpel, S., von Blanckenhagen, F., Maaz, D., Herden, C., Freise, J., Wolf, R., Stubbe, M., Borkenhagen, P., Ansorge, H., Eccard, J. A., Lang, J., Jourdain, E., Jacob, J., Marianneau, P., Heckel, G. and Ulrich, R. G. (2016). High genetic structuring of Tula hantavirus. Arch Virol *161 (5)*, 1135-1149, doi: 10.1007/s00705-016-2762-6.

Schötta, A. M., Wijnveld, M., Stockinger, H. and Stanek, G. (2017). Approaches for Reverse Line Blot-Based Detection of Microbial Pathogens in Ixodes ricinus Ticks Collected in Austria and Impact of the Chosen Method. Appl Environ Microbiol *83 (13)*, doi: 10.1128/aem.00489-17.

Servan, J., Brault, J., Alonso, J. M., Bercovier, H. and Mollaret, H. H. (1979). *Yersinia enterocolitica* among small wild mammals in France. Comp Immunol Microbiol Infect Dis *1 (4)*, 321-333.

Sharp, P. M. and Hahn, B. H. (2011). Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med *1 (1)*, a006841, doi: 10.1101/cshperspect.a006841.

Shchelkunov, S. N. (2009). How long ago did smallpox virus emerge? Arch Virol *154 (12)*, 1865-1871, doi: 10.1007/s00705-009-0536-0.

Shi, M., Lin, X. D., Chen, X., Tian, J. H., Chen, L. J., Li, K., Wang, W., Eden, J. S., Shen, J. J., Liu, L., Holmes, E. C. and Zhang, Y. Z. (2018). The evolutionary history of vertebrate RNA viruses. Nature *556 (7700)*, 197-202, doi: 10.1038/s41586-018-0012-7.

Sibold, C., Meisel, H., Krüger, D. H., Labuda, M., Lysy, J., Kozuch, O., Pejcoch, M., Vaheri, A. and Plyusnin, A. (1999a). Recombination in Tula hantavirus evolution: analysis of genetic lineages from Slovakia. J Virol *73 (1)*, 667-675.

Sibold, C., Meisel, H., Lundkvist, Ä., Schulz, A., Cifire, F., Ulrich, R., Kozuch, O., Labuda, M. and Krüger, D. H. (1999b). SHORT REPORT: SIMULTANEOUS OCCURRENCE OF DOBRAVA, PUUMALA, AND TULA HANTAVIRUSES IN SLOVAKIA. Am J Trop Med Hyg *61 (3)*, 409-411, doi: 10.4269/ajtmh.1999.61.409.

Sibold, C., Ulrich, R., Labuda, M., Lundkvist, Ä., Martens, H., Schutt, M., Gerke, P., Leitmeyer, K., Meisel, H. and Krüger, D. H. (2001). Dobrava hantavirus causes hemorrhagic fever with renal syndrome in central Europe and is carried by two different *Apodemus* mice species. J Med Virol *63 (2)*, 158-167.

Sinski, E., Bajer, A., Welc, R., Pawelczyk, A., Ogrzewalska, M. and Behnke, J. M. (2006). *Babesia microti:* prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes District of North-Eastern Poland. Int J Med Microbiol *296 Suppl 40*, 137-143, doi: 10.1016/j.ijmm.2006.01.015.

Sironen, T., Vaheri, A. and Plyusnin, A. (2001). Molecular evolution of Puumala hantavirus. J Virol *75 (23)*, 11803-11810, doi: 10.1128/jvi.75.23.11803-11810.2001.

Smith, D. J. and Self, H. R. (1955). Observations on the Survival of *Leptospira Australis* A in Soil and Water. J Hyg (Lond) *53 (4)*, 436-444.

Smith, K. M., Machalaba, C. C., Seifman, R., Feferholtz, Y. and Karesh, W. B. (2019). Infectious disease and economics: The case for considering multi-sectoral impacts. One Health *7*, 100080, doi: 10.1016/j.onehlt.2018.100080.

Song, J. W., Gligic, A. and Yanagihara, R. (2002). Identification of Tula hantavirus in *Pitymys subterraneu*s captured in the Cacak region of Serbia-Yugoslavia. Int J Infect Dis *6 (1)*, 31-36.

Song, J. W., Baek, L. J., Song, K. J., Skrok, A., Markowski, J., Bratosiewicz-Wasik, J., Kordek, R., Liberski, P. P. and Yanagihara, R. (2004). Characterization of Tula Virus from Common Voles (*Microtus Arvalis*) in Poland: Evidence for Geographic-Specific Phylogenetic Clustering. Virus Genes *29 (2)*, 239-247, doi: 10.1023/B:VIRU.00000 36384.50102.cf.

Song, J. W., Gu, S. H., Bennett, S. N., Arai, S., Puorger, M., Hilbe, M. and Yanagihara, R. (2007). Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew (*Sorex araneus*). Virol J *4*, 114, doi: 10.1186/1743-422x-4-114.

Song, K. J., Baek, L. J., Moon, S., Ha, S. J., Kim, S. H., Park, K. S., Klein, T. A., Sames, W., Kim, H. C., Lee, J. S., Yanagihara, R. and Song, J. W. (2007). Muju virus, a novel hantavirus harboured by the arvicolid rodent *Myodes regulus* in Korea. J Gen Virol *88 (Pt 11)*, 3121-3129, doi: 10.1099/vir.0.83139-0.

Souza, W. M., Bello, G., Amarilla, A. A., Alfonso, H. L., Aquino, V. H. and Figueiredo, L. T. (2014). Phylogeography and evolutionary history of rodent-borne hantaviruses. Infect Genet Evol *21*, 198-204, doi: 10.1016/j.meegid.2013.11.015.

Spichler, A. S., Vilaca, P. J., Athanazio, D. A., Albuquerque, J. O., Buzzar, M., Castro, B., Seguro, A. and Vinetz, J. M. (2008). Predictors of lethality in severe leptospirosis in urban Brazil. Am J Trop Med Hyg *79 (6)*, 911-914.

Spitzenberger, F. (1997). [First record of the Striped Field Mouse (*Apodemus agrarius*, Muridae, Mammalia) in Lower Austria] [in German]. Mamm Biol *62*, 250-252.

Spitzenberger, F. and Engelberger, S. (2014). A new look at the dynamic western distribution border of Apodemus agrarius in Central Europe (Rodentia: Muridae). Lnynx series nova *45*, 69-79.

Stanojevic, M., Nikolic, V., Stajkovic, N., Stamenkovic, G., Bozovic, B., Cekanac, R., Marusic, P. and Gligic, A. (2015). Genetic detection of Dobrava-Belgrade hantavirus in the edible dormouse (*Glis glis*) in central Serbia. Epidemiol Infect *143 (2)*, 400-404, doi: 10.1017/s0950268814001010.

Straková, P., Dufkova, L., Širmarová, J., Salát, J., Bartonička, T., Klempa, B., Pfaff, F., Höper, D., Hoffmann, B., Ulrich, R. G. and Růžek, D. (2017). Novel hantavirus identified in European bat species *Nyctalus noctula*. Infect Genet Evol *48*, 127-130, doi: 10.1016/j.meegid.2016.12.025.

Stritof Majetic, Z., Galloway, R., Ruzic Sabljic, E., Milas, Z., Mojcec Perko, V., Habus, J., Margaletic, J., Pernar, R. and Turk, N. (2014). Epizootiological survey of small mammals as Leptospira spp. reservoirs in Eastern Croatia. Acta Trop *131*, 111-116, doi: 10.1016/j.actatropica.2013.12.009.

Sunil-Chandra, N. P., Clement, J., Maes, P.,De Silva, H. J., Van Esbroeck, M. and Van Ranst, M. (2015). Concomitant leptospirosis-hantavirus co-infection in acute patients hospitalized in Sri Lanka: implications for a potentially worldwide underestimated problem. Epidemiol Infect *143 (10)*, 2081-2093, doi: 10.1017/s0950268814003707.

Svitalkova, Z. H., Harustiakova, D., Mahrikova, L., Mojsova, M., Berthova, L., Slovak, M., Kocianova, E., Vayssier-Taussat, M. and Kazimirova, M. (2016). *Candidatus* Neoehrlichia mikurensis in ticks and rodents from urban and natural habitats of South-Western Slovakia. Parasit Vectors *9*, 2, doi: 10.1186/s13071-015-1287-2.

Szekeres, S., Claudia Coipan, E., Rigo, K., Majoros, G., Jahfari, S., Sprong, H. and Foldvari, G. (2015). *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* in natural rodent and tick communities in Southern Hungary. Ticks Tick Borne Dis *6 (2)*, 111-116, doi: 10.1016/j.ttbdis.2014.10.004.

Tadin, A., Turk, N., Korva, M., Margaletic, J., Beck, R., Vucelja, M., Habus, J., Svoboda, P., Zupanc, T. A., Henttonen, H. and Markotic, A. (2012). Multiple co-infections of rodents with hantaviruses, *Leptospira*, and *Babesia* in Croatia. Vector Borne Zoonotic Dis *12 (5)*, 388-392, doi: 10.1089/vbz.2011.0632.

Tadin, A., Tokarz, R., Markotic, A., Margaletic, J., Turk, N., Habus, J., Svoboda, P., Vucelja, M., Desai, A., Jain, K. and Lipkin, W. I. (2016). Molecular Survey of Zoonotic Agents in Rodents and Other Small Mammals in Croatia. Am J Trop Med Hyg *94 (2)*, 466-473, doi: 10.4269/ajtmh.15-0517.

Tagliapietra, V., Rosa, R., Hauffe, H. C., Laakkonen, J., Voutilainen, L., Vapalahti, O., Vaheri, A., Henttonen, H. and Rizzoli, A. (2009). Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, northern Italy. Emerg Infect Dis *15 (7)*, 1019-1025, doi: 10.3201/eid1507.081524.

Taller, A. M., Xiao, S. Y., Godec, M. S., Gligic, A., Avsic-Zupanc, T., Goldfarb, L. G., Yanagihara, R. and Asher, D. M. (1993). Belgrade virus, a cause of hemorrhagic fever with renal syndrome in the Balkans, is closely related to Dobrava virus of field mice. J Infect Dis *168 (3)*, 750-753, doi: 10.1093/infdis/168.3.750.

Taylor, L. H., Latham, S. M. and Woolhouse, M. E. (2001). Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci *356 (1411)*, 983-989, doi: 10.1098/rstb.2001.0888.

Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S. and Begon, M. (2010). Species interactions in a parasite community drive infection risk in a wildlife population. Science *330 (6001)*, 243-246, doi: 10.1126/science.1190333.

Thibeaux, R., Girault, D., Bierque, E., Soupe-Gilbert, M. E., Rettinger, A., Douyere, A., Meyer, M., Iraola, G., Picardeau, M. and Goarant, C. (2018a). Biodiversity of Environmental *Leptospira*: Improving Identification and Revisiting the Diagnosis. Front Microbiol *9*, 816, doi: 10.3389/fmicb.2018.00816.

Thibeaux, R., Iraola, G., Ferrés, I., Bierque, E., Girault, D., Soupé-Gilbert, M. E., Picardeau, M. and Goarant, C. (2018b). Deciphering the unexplored Leptospira diversity from soils uncovers genomic evolution to virulence. Microb Genom *4 (1)*, doi: 10.1099/mgen.0.000144.

Thiermann, A. B. (1981). The Norway rat as a selective chronic carrier of Leptospira icterohaemorrhagiae. J Wildl Dis *17 (1)*, 39-43, doi: 10.7589/0090-3558-17.1.39.

Thoma, B. R., Müller, J., Bässler, C., Georgi, E., Osterberg, A., Schex, S., Bottomley, C. and Essbauer, S. S. (2014). Identification of FactorsInfluencing the Puumala Virus Seroprevalence within Its Reservoir in aMontane Forest Environment. Viruses *6 (10)*, 3944-3967, doi: 10.3390/v6103944.

Thomason, A. G., Begon, M., Bradley, J. E., Paterson, S. and Jackson, J. A. (2017). Endemic Hantavirus in Field Voles, Northern England. Emerg Infect Dis *23 (6)*, 1033-1035, doi: 10.3201/eid2306.161607.

Tkachenko, E. A., Okulova, N. M., Iunicheva, I., V., Morzunov, S. P., Khaibulina, S. F., Riabova, T. E., Vasilenko, L. E., Bashkirtsev, V. N., Dzagurova, T. K., Gorbachkova, E. A., Sedova, N. S., Balakirev, A. E., Dekonenko, A. E. and Drozdov, S. G. (2005). [The epizootological and virological characteristics of a natural hantavirus infection focus in the subtropic zone of the Krasnodarsk Territory] [in Russian]. Vopr Virusol *50 (3)*, 14-19.

Tkachenko, E. A., Witkowski, P. T., Radosa, L., Dzagurova, T. K., Okulova, N. M., Yunicheva, Y. V., Vasilenko, L., Morozov, V. G., Malkin, G. A., Krüger, D. H. and Klempa, B. (2015). Adler hantavirus, a new genetic variant of Tula virus identified in Major's pine voles (*Microtus majori*) sampled in southern European Russia. Infect Genet Evol *29*, 156-163, doi: 10.1016/j.meegid.2014.11.018.

Tobudic, S., Burgmann, H., Stanek, G., Winkler, S., Schötta, A. M., Obermüller, M., Markowicz, M. and Lagler, H. (2020). Human *Borrelia miyamotoi* Infection, Austria. Emerg Infect Dis *26 (9)*, 2201-2204, doi: 10.3201/eid2609.191501.

Tolkacz, K., Bednarska, M., Alsarraf, M., Dwuznik, D., Grzybek, M., Welc-Faleciak, R., Behnke, J. M. and Bajer, A. (2017). Prevalence, genetic identity and vertical transmission of *Babesia microti* in three naturally infected species of vole, *Microtus* spp. (Cricetidae). Parasit Vectors *10 (1)*, 66, doi: 10.1186/s13071-017-2007-x.

Tolkacz, K., Alsarraf, M., Kowalec, M., Dwuznik, D., Grzybek, M., Behnke, J. M. and Bajer, A. (2018). *Bartonella* infections in three species of *Microtus*: prevalence and genetic diversity, vertical transmission and the effect of concurrent *Babesia microti* infection on its success. Parasit Vectors *11 (1)*, 491, doi: 10.1186/s13071-018-3047-6.

Torres-Castro, M., Guillermo-Cordero, L., Hernandez-Betancourt, S., Gutierrez-Ruiz, E., Agudelo-Florez, P., Pelaez-Sanchez, R., Zavala-Castro, J. and Puerto, F. I. (2016). First histopathological study in kidneys of rodents naturally infected with *Leptospira* pathogenic species from Yucatan, Mexico. Asian Pac J Trop Med *9 (2)*, 145-147, doi: 10.1016/j.apjtm.2016.01.018.

Trueba, G., Zapata, S., Madrid, K., Cullen, P. and Haake, D. (2004). Cell aggregation: a mechanism of pathogenic *Leptospira* to survive in fresh water. Int Microbiol *7 (1)*, 35-40.

Vaheri, A., Henttonen, H., Voutilainen, L., Mustonen, J., Sironen, T. and Vapalahti, O. (2013a). Hantavirus infections in Europe and their impact on public health. Rev Med Virol *23 (1)*, 35-49, doi: 10.1002/rmv.1722.

Vaheri, A., Strandin, T., Hepojoki, J., Sironen, T., Henttonen, H., Makela, S. and Mustonen, J. (2013b). Uncovering the mysteries of hantavirus infections. Nat Rev Microbiol *11 (8)*, 539-550.

Vanasco, N., Sequeira, M., Sequeira, G. and Tarabla, H. (2003). Associations between leptospiral infection and seropositivity in rodents and environmental characteristics in Argentina. Prev Vet Med *60 (3)*, 227-235.

Vapalahti, O., Lundkvist, Ä., Fedorov, V., Conroy, C. J., Hirvonen, S., Plyusnina, A., Nemirov, K., Fredga, K., Cook, J. A., Niemimaa, J., Kaikusalo, A., Henttonen, H., Vaheri, A. and Plyusnin, A. (1999). Isolation and characterization of a hantavirus from *Lemmus sibiricus*: evidence for host switch during hantavirus evolution. J Virol *73 (7)*, 5586-5592.

Vidal, D., Alzaga, V., Luque-Larena, J. J., Mateo, R., Arroyo, L. and Vinuela, J. (2009). Possible interaction between a rodenticide treatment and a pathogen in common vole (*Microtus arvalis*) during a population peak. Sci Total Environ *408 (2)*, 267-271, doi: 10.1016/j.scitotenv.2009.10.001.

Vijayachari, P., Sugunan, A. P. and Shriram, A. N. (2008). Leptospirosis: an emerging global public health problem. J Biosci *33 (4)*, 557-569, doi: 10.1007/s12038-008-0074-z.

Vitale, M., Agnello, S., Chetta, M., Amato, B., Vitale, G., Bella, C. D., Vicari, D. and Presti, V. (2018). Human leptospirosis cases in Palermo Italy. The role of rodents and climate. J Infect Public Health *11 (2)*, 209-214, doi: 10.1016/j.jiph.2017.07.024.

Vollmar, P., Lubnow, M., Simon, M., Muller, T., Bergler, T., Alois, P., Thoma, B. R. and Essbauer, S. (2016). Hantavirus cardiopulmonary syndrome due to Puumala virus in Germany. J Clin Virol *84*, 42-47, doi: 10.1016/j.jcv.2016.10.004.

Voutilainen, L., Savola, S., Kallio, E. R., Laakkonen, J., Vaheri, A., Vapalahti, O. and Henttonen, H. (2012). Environmental change and disease dynamics: effects of intensive forest management on Puumala hantavirus infection in boreal bank vole populations. PLoS One *7 (6)*, e39452, doi: 10.1371/journal.pone.0039452.

Voutilainen, L., Sironen, T., Tonteri, E., Bäck, A. T., Razzauti, M., Karlsson, M., Wahlström, M., Niemimaa, J., Henttonen, H. and Lundkvist, Ä. (2015). Life-long shedding of Puumala hantavirus in wild bank voles (*Myodes glareolus*). J Gen Virol *96 (Pt 6)*, 1238-1247, doi: 10.1099/vir.0.000076.

Voutilainen, L., Kallio, E. R., Niemimaa, J., Vapalahti, O. and Henttonen, H. (2016). Temporal dynamics of Puumala hantavirus infection in cyclic populations of bank voles. Sci Rep *6*, 21323, doi: 10.1038/srep21323.

Vyrostekova, V., Khanakah, G., Kocianova, E., Gurycova, D. and Stanek, G. (2002). Prevalence of coinfection with *Francisella tularensis* in reservoir animals of *Borrelia burgdorferi* sensu lato. Wien Klin Wochenschr *114 (13-14)*, 482-488.

Ward, M. P. (2002). Seasonality of canine leptospirosis in the United States and Canada and its association with rainfall. Prev Vet Med *56 (3)*, 203-213, doi: 10.1016/s0167-5877(02)00183-6.

Weber de Melo, V., Sheikh Ali, H., Freise, J., Kuhnert, D., Essbauer, S., Mertens, M., Wanka, K. M., Drewes, S., Ulrich, R. G. and Heckel, G. (2015). Spatiotemporal dynamics of Puumala hantavirus associated with its rodent host, *Myodes glareolus*. Evol Appl *8 (6)*, 545-559, doi: 10.1111/eva.12263.

Webster, J. P., Gower, C. M., Knowles, S. C., Molyneux, D. H. and Fenton, A. (2016). One health–an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases. Evol Appl *9 (2)*, 313-333.

Weidmann, M., Schmidt, P., Vackova, M., Krivanec, K., Munclinger, P. and Hufert, F. T. (2005). Identification of Genetic Evidence for Dobrava Virus Spillover in Rodents by Nested Reverse Transcription (RT)-PCR and TaqMan RT-PCR. J Clin Microbiol *43 (2)*, 808-812, doi: 10.1128/jcm.43.2.808-812.2005.

White, T. A., Perkins, S. E., Heckel, G. and Searle, J. B. (2013). Adaptive evolution during an ongoing range expansion: the invasive bank vole (*Myodes glareolus*) in Ireland. Mol Ecol *22 (11)*, 2971-2985, doi: 10.1111/mec.12343.

Wilcove, D. S., Giam, X., Edwards, D. P., Fisher, B. and Koh, L. P. (2013). Navjot's nightmare revisited: logging, agriculture, and biodiversity in Southeast Asia. Trends Ecol Evol *28 (9)*, 531-540, doi: 10.1016/j.tree.2013.04.005.

Wilson, D. E., Mittermeier, R. A., Ruff, S., Martínez-Vilalta, A. and Cavallini, P. (2016). Handbook of the Mammals of the World: Lagomorphs and Rodents I. Lynx Edicions. Barcelona

Wójcik-Fatla, A., Zając, V., Knap, J. P., Sroka, J., Cisak, E., Sawczyn, A. and Dutkiewicz, J. (2013). A small-scale survey of hantavirus in mammals from eastern Poland. Ann Agric Environ Med *20 (2)*, 283-286.

World Health Organization (2020). Zoonoses. URL: https://www.who.int/zoonoses/en/ [as of 22.08.2019].

Wynwood, S. J., Graham, G. C., Weier, S. L., Collet, T. A., McKay, D. B. and Craig, S. B. (2014). Leptospirosis from water sources. Pathog Glob Health *108 (7)*, 334-338, doi: 10.1179/2047773214y.0000000156.

Xu, Y., Zhu, Y., Wang, Y., Chang, Y. F., Zhang, Y., Jiang, X., Zhuang, X., Zhu, Y., Zhang, J., Zeng, L., Yang, M., Li, S., Wang, S., Ye, Q., Xin, X., Zhao, G., Zheng, H., Guo, X. and Wang, J. (2016). Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic *Leptospira*. Sci Rep *6*, 20020, doi: 10.1038/srep20020.

Yanagihara, R., Svedmyr, A., Amyx, H. L., Lee, P., Goldgaber, D., Gajdusek, D. C., Gibbs, C. J., Jr. and Nyström, K. (1984). Isolation and propagation of nephropathia epidemica virus in bank voles. Scand J Infect Dis *16 (3)*, 225-228, doi: 10.3109/00365548409070393.

Yashina, L. N., Abramov, S. A., Dupal, T. A., Danchinova, G. A., Malyshev, B. S., Hay, J., Gu, S. H. and Yanagihara, R. (2015). Hokkaido genotype of Puumala virus in the grey red-backed vole (*Myodes rufocanus*) and northern red-backed vole (*Myodes rutilus*) in Siberia. Infect Genet Evol *33*, 304-313, doi: 10.1016/j.meegid.2015.05.021.

Yigit, N., Hutterer, R., Krytufek, B. & Amori, G. 2016 (2016). *Microtus arvalis*. URL: https://www.iucnredlist.org/species/13488/22351133 [as of 24.05.2020].

Zelena, H., Mrazek, J. and Kuhn, T. (2013). Tula hantavirus infection in immunocompromised host, Czech Republic. Emerg Infect Dis *19 (11)*, 1873-1875, doi: 10.3201/eid1911.130421.

Zhang, F., Gong, Z., Zhang, J. and Liu, Z. (2010). Prevalence of *Borrelia burgdorferi* sensu lato in rodents from Gansu, northwestern China. BMC Microbiol *10*, 157, doi: 10.1186/1471-2180-10-157.

Appendix

Appendix 1: Supplementary tables

Table S1: Fungi detected in common voles

Order	Family	Genus	Pathogen	Prevalence**	Reference
Microsporida	Cryptosporidiidae	*Cryptosporidium*	*Encephalitozoon cuniculi*	6.0%	(Führer et al. 2010)
Onygenales	Arthrodermataceae	*Trichophyton*	*Trichophyton mentagrophytes*	5.0%*	(Chmel et al. 1975)
	Onygenaceae	*Emmonsia*	*Emmonsia crescens/ Adiaspores*	2.7-7.3%*	(Hubalek 1999; Hubalek et al. 1995)

* microscopical/histological detection; ** prevalence is given if at least 10 common voles were tested

Table S2: Viruses that were detected in common voles

Realm	Kingdom	Phylum	Class	Order	Family	Genus	Pathogen	Prevalence**	Reference
Monodnaviria	*Shotokuvirae*	*Cossaviricota*	*Papovaviricetes*	*Sepolvirales*	*Polyomaviridae*	*Betapolyomavirus*	Microtus arvalis polyomavirus 1	12.9%	(Nainys et al. 2015)
Riboviria	*Orthornavirae*	*Duplornaviricota*	*Resentoviricetes*	*Reovirales*	*Reoviridae* Subfamily: *Spinareovirinae*	*Orthoreovirus*	Mammalian orthoreovirus novel strain	n.a.	(Feher et al. 2017)
		Kitrinoviricota	*Alsuviricetes*	*Hepelivirales*	*Hepeviridae*	*Orthohepevirus*	Orthohepevirus C (Common vole associated Hepatitis E Virus)	2.0-10.3%	(Kurucz et al. 2019; Ryll et al. 2019)
			Flasuviricetes	*Amarillovirales*	*Flaviviridae*	*Flavivirus*	Tick-borne encephalitis virus	6.3-9.5%	(Achazi et al. 2011; Pinter et al. 2014)
		Negarnaviricota Subphylum: *Polyploviricotina*	*Ellioviricetes*	*Bunyavirales*	*Arenaviridae*	*Mammarenavirus*	Lymphocytic choriomeningitis mammarenavirus	14.3-25.0%*	(Kallio-Kokko et al. 2006; Tagliapietra et al. 2009)
					Hantaviridae Subfamily: *Mammantavirinae*	*Orthohantavirus*	Tula orthohantavirus	11.8-37.8%	(see Table 2, PAPER I, IV)
					Peribunyaviridae	*Orthobunyavirus*	California encephalitis orthobunyavirus	80.0%*	(Mitchell et al. 1993)
		Pisuviricota	*Pisoniviricetes*	*Picornavirales*	*Picornaviridae*	*Cardiovirus*	Cardiovirus A	8.3%*	(Billinis 2009)
Varidnaviria	*Bamfordvirae*	*Nucleocytoviricota*	*Pokkesviricetes*	*Chitovirales*	*Poxviridae* Subfamily: *Chordopoxvirinae*	*Orthopoxvirus*	Cowpox virus	7.1%	(Prkno et al. 2017)

n.a., not available; * serological detection; ** prevalence is given for studies with at least 10 tested common voles

Table S3   Bacteria that were detected in common voles

Order	Family	Genus	Pathogen	Prevalence**	Reference
Actinomycetales	Mycobacteriaceae	Mycobacterium	Mycobacterium chelonae	n.a.	(Fischer et al. 2000)
			Mycobacterium fortuitum	n.a.	(Fischer et al. 2000)
Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus microti	n.a.	(Novakova et al. 2010)
Chlamydiales	Chlamydiaceae	Chlamydia	Chlamydophila psittaci	12.0%*	(Cislakova et al. 2004)
Desulfovibrionales	Desulfovibrionaceae	Lawsonia	Lawsonia intracellularis	n.a.	(Friedman et al. 2008)
Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia enterocolitica	4.2%	(Servan et al. 1979)
			Yersina pestis biovar Microtus	n.a.	(Kislichkina et al. 2017)
Legionellales	Coxiellaceae	Coxiella	Coxiella burnetii	n.a.*	(Literak 1995)
Pasteurellales	Pasteurellaceae	Pasteurella	Pastorella pneumotropica	n.a.	(Boot et al. 1986)
Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas stutzeri	n.a.	(Hubalek et al. 1998)
Rhizobiales	Bartonellaceae	Bartonella	Bartonella doshiae	4.2%	(Paziewska et al. 2011; Rodriguez-Pastor et al. 2018)
			Bartonella grahamii	6.3%	(Paziewska et al. 2011; Rodriguez-Pastor et al. 2018; Tolkacz et al. 2018)
			Bartonella rochalimae-like	9.2%	(Rodriguez-Pastor et al. 2018; Tolkacz et al. 2018)
			Bartonella taylorii	13.3-30.0%	(Paziewska et al. 2011; Rodriguez-Pastor et al. 2018; Schmidt et al. 2014; Tolkacz et al. 2018)
	Brucellaceae	Brucella	Brucella microti	n.a.	(Hubalek et al. 2007)
Rickettsiales	Anaplasmataceae	Anaplasma	Anaplasma phagocytophilum	1.6%	(Matei et al. 2018; Szekeres et al. 2015)
		Neoehrlichia	Neoehrlichia mikurensis	30.0%	(Svitalkova et al. 2016)
	Rickettsiaceae	Rickettsia	Rickettsia helvetica	10.0%	(Heglasova et al. 2018)
			Rickettsia raoultii	0.3%	(Fischer et al. 2018b)
	Pseudomonadaceae	Pseudomonas	Pseudomonas stutzeri	n.a.	(Hubalek et al. 1998)
Spirochaetales	Leptospiraceae	Leptospira	Leptospira kirschneri	6.7-30.0%	(Fischer et al. 2018a; Mayer-Scholl et al. 2014; Schmidt et al. 2014, PAPER III, PAPER IV)
	Spirochaetaceae	Borrelia	Borrelia afzelii	n.a.	(Khanakah et al. 2006)
			Borrelia burgdorferi s.l.	20.7-68.4%	{Vyrostekova, 2002 #67;Hamsikova, 2016 #220}
			Borrelia garinii	6.7%	(Schmidt et al. 2014)
Thiotrichales	Francisellaceae	Francisella	Francisella tularensis	3.0-32.2%	(Elashvili et al. 2015; Jeske et al. 2019a; Rodriguez-Pastor et al. 2017; Vidal et al. 2009)

n.a., not available, * serological detection; **prevalence is given if at least 10 common voles were tested

Table S4 Parasites that were detected in common voles

Order	Family	Genus	Pathogen	Prevalence**	Reference
Eukaryota	Diplomonadida	Hexamitidae	*Giardia*	*Giardia* spp.	10.3-96.3%*	(Bajer 2008; Bajer et al. 2002, Helmy et al. 2018, Riebold et al., 2020)
	Eucoccidiorida	Cryptosporidiidae	*Cryptosporidium*	*Cryptosporidium parvum*	62.3%*	(Bajer 2008)
				Cryptosporidium spp.	3.4%	(Riebold et al., 2020)
		Eimeriidae	*Eimeria*	*Eimeria* spp.	51.7%	(Riebold et al., 2020)
			Cyclospora	*Cyclospora* spp.	6.9%	(Riebold et al., 2020)
		Hepatozoidae	*Hepatozoon*	*Hepatozoon lavieri*	3.1%*	(Pawelczyk et al. 2004)
		Sarcocystidae	*Neospora*	*Neospora caninum*	1.5%	(Führer et al. 2010)
			Sarcocystis	*Sarcocystis microti*	8.2%*	(Fichet-Calvet et al. 2004)
				Sarcocystis cernae	10.3%	(Hoogenboom and Dijkstra 1987)
				Sarcocystis spp.	24.1%	(Riebold et al., 2020)
			Toxoplasma	*Toxoplasma gondii*	0.7%	(Führer et al. 2010)
	Haemosporida	Babesiidae	*Babesia*	*Babesia microti*	45.2-71.7%	(Sinski et al. 2006; Tolkacz et al. 2017)
	Retortamonadida	Retortamonadidae	*Chilomastix*	*Chilomastix* spp.	3.4%	(Riebold et al., 2020)
	Trypanosomatida	Trypanosomatidae	*Trypanosoma*	*Trypanosoma* spp.	8.4%*	(Pawelczyk et al. 2004)
Nematoda	Enoplea	Capillariidae	*Capillaria*	*Calodium hepaticum/ Capillaria hepatica* (syn.)	0.9%*	(Führer et al. 2010)
	Oxyurida	Oxyuridae	*Aspiculuris*	*Aspiculuris tetraptera*	3.4%	(Riebold et al., 2020)
			Syphacia	*Syphacia obvelata*	20.7%	(Riebold et al., 2020)
	Rhabditida	Trychostrongylidae	*Heligmosomoides*	*Heligmosomoides polygyrus*, (previously named: *Nematospiroides dubius*)	20.7%	(Riebold et al., 2020)
			Trichostrongylus	*Trichostrongylus* spp.	3.4%	(Riebold et al., 2020)
	Strongylida	Heligmosomidae	*Heligmosomoides*	*Heligmosomum costellatum*	27.6%*	(Janova et al. 2010)
	Trichocephalida	Trichinellidae	*Trichinella*	*Trichuris arvicolae*	9.9%*	(Feliu et al. 2000)
				Trichuris spp.	6.9%	(Riebold et al., 2020)
Plathelminthes	Cyclophyllidea	Hymenolepididae	*Hymenolepis*	*Hymenolepis diminuta*	24.1	(Riebold et al., 2020)
			Rodentolepis	*Rodentolepis asymmetrica*	1%	(Führer et al. 2010)
		Mesocestoididae	*Mesocestoides*	*Mesocestoides leptothylacus*	1.4%*	(Loos-Frank 1980)
		Taeniidae	*Echinococcus*	*Echinococcus multilocularis*	0.1-18.6%	(Gottstein et al. 2001; Hanosset et al. 2008)
			Taenia	*Taenia taeniaeformis*	1.0%	(Führer et al. 2010)

*serological or microscopical detection, ** prevalence is given if at least 10 common voles were tested

Appendix 2:   Own contribution to publications

Publication I: Field vole-associated Traemmersee hantavirus from Germany represents a novel hantavirus species

Jeske K, Hiltbrunner M, Drewes S, Ryll R, Wenk M, Špakova A, Petraityte-Burneikiene R, Heckel G, and Ulrich RG

Jeske K:	Dissection of small mammals
	RNA and DNA extraction
	Screening of all samples by RT-PCR and ELISA
	Cytochrome b-PCR
	Generation of a new TULV antigen
	Primer walking of S, M and L segment of TRAV
	Phylogenetic tree reconstruction
	Writing of manuscript
	Proofreading of manuscript
Hiltbrunner M	Hybrid sequence capture enrichment in combination with high-throughput sequencing for TRAV
	Proofreading of manuscript
Drewes S	Dissection of small mammals
	Proofreading of manuscript
Ryll R	Dissection of small mammals
	Pairwise evolutionary distance analysis
	Proofreading of manuscript
Wenk M	Small mammal trapping coordination
	Proofreading of manuscript
Špakova A	Generation of a new TULV antigen
	Proofreading of manuscript
Petraityte-Burneikiene R	Supervision for generation of the new antigen
	Proofreading of manuscript

Heckel G   Supervision of hybrid sequence capture enrichment in combination with high-throughput sequencing for TRAV
Writing of manuscript
Proofreading of manuscript

Ulrich RG   Study design
Writing of manuscript
Proofreading of manuscript

Publication II: Identification of a novel hantavirus strain in the root vole (*Microtus oeconomus*) in Lithuania, Eastern Europe

Drewes S*, **Jeske K***, Straková P, Balčiauskas L, Ryll R, Balčiauskienė L, Kohlhause D, Schnidrig G, Hiltbrunner M, Špakova A, Insodaitė R, Petraitytė-Burneikienė R, Heckel G, and Ulrich RG

*contributed equally

Drewes S*	Screening of some samples by RT-PCR
	Primer walking analysis
	Phylogenetic tree reconstruction
	Writing of manuscript
	Proofreading of manuscript
Jeske K*	Screening of some samples by RT-PCR
	Generation of RUSV antigen
	Screening of all samples by ELISA
	Primer walking analysis
	Phylogenetic tree reconstruction
	Writing of manuscript
	Proofreading of manuscript
Straková P	Screening of some samples by RT-PCR
	Primer walking analysis
	Proofreading of manuscript
Balčiauskas L	Trapping of small mammals and dissection
	Proofreading of manuscript
Ryll R	Pairwise evolutionary distance analysis
	Proofreading of manuscript
Balčiauskienė L	Trapping of small mammals and dissection
	Proofreading of manuscript
Kohlhause D	Primer walking analysis
	Proofreading of manuscript

Schnidrig G	Hybrid sequence capture enrichment in combination with high-throughput sequencing for RUSV Proofreading of manuscript
Hiltbrunner M	Hybrid sequence capture enrichment in combination with high-throughput sequencing for RUSV Proofreading of manuscript
Špakova A	Generation of RUSV antigen Testing of RUSV antigen Proofreading of manuscript
Insodaitė R	Testing of RUSV antigen Proofreading of manuscript
Petraitytė-Burneikienė R	Supervision of generation of RUSV antigen Proofreading of manuscript
Heckel G	Supervision of hybrid sequence capture enrichment in combination with high-throughput sequencing for RUSV Proofreading of manuscript
Ulrich RG	Study design Proofreading of manuscript

Publication III: Multiple *Leptospira* spp. detection, but absence of Tula orthohantavirus in *Microtus* voles, Northwestern Spain

Jeske K, Emirhar D, García JT, González-Barrio D, Olea PP, Ruiz Fons F, Schulz J, Mayer-Scholl A, Heckel G, and Ulrich RG

Jeske K	RNA and DNA extraction
	Screening of samples
	Writing of manuscript
	Proofreading of manuscript
Emirhar D	Partial screening for *Leptospira* spp.
	Partial *sec*Y and MLST PCR
	Proofreading of manuscript
García JT	Proofreading of manuscript
González-Barrio D	Proofreading of manuscript
Olea PP	Statistical model calculation
	Proofreading of manuscript
Ruiz Fons F	Coordination of sample collection
	Proofreading of manuscript
Schulz J	Statistical model calculation
	Supervision of statistical model calculations
	Proofreading of manuscript
Mayer-Scholl A	Supervision of *Leptospira* spp. typing
	Proofreading of manuscript
Heckel G	Supervision of phylogenetic investigation
	Proofreading of manuscript
Ulrich RG	Study design
	Writing of manuscript
	Proofreading of manuscript

Publication IV: Hantavirus - *Leptospira* coinfections in small mammals from central Germany

Jeske K, Jacob J, Drewes S, Pfeffer M, Heckel G, Ulrich RG, and Imholt C

Jeske K	Trapping and dissection of small mammals
	DNA and RNA extraction
	Screening of all samples
	Phylogenetic tree reconstruction
	Writing of manuscript
	Proofreading of manuscript
Jacob J	Study design
	Proofreading of manuscript
Drewes S	Dissection of small mammals
	Proofreading of manuscript
Pfeffer M	Proofreading of manuscript
Heckel G	Proofreading of manuscript
Ulrich RG	Writing of manuscript
	Proofreading of manuscript
Imholt C	Study design
	Trapping of small mammals
	Statistical analysis
	Writing of manuscript
	Proofreading of manuscript

Publication V: Zoonotic pathogen screening in striped field mouse (*Apodemus agrarius*) from Austria

Jeske K, Herzig-Straschil B, Raileanu C, Kunec D, Tauchmann O, Emirhar D, Schmidt S, Trimpert J, Silaghi C, Heckel G, Ulrich RG and Drewes S

Jeske K	Dissection of small mammals
	DNA extraction and *Leptospira* screening
	Primer walking analysis
	Phylogenetic tree reconstruction
	Proofreading of manuscript
Herzig-Straschil B	Collection of striped field mice
	Proofreading of manuscript
Raileanu C	Extraction of DNA
	Screening for vector-borne pathogens
	Proofreading of manuscript
Kunec D	Next-generation sequencing
	Proofreading of manuscript
Tauchmann O	Extraction of DNA
	Screening for vector-borne pathogens
	Proofreading of manuscript
Emirhar D	*sec*Y and MLST-PCR
	Proofreading of manuscript
Schmidt S	RNA extraction of some lung tissues
	Screening of some samples for DOBV
	Proofreading of manuscript
Trimpert J	Supervision of next-generation sequencing
	Proofreading of manuscript
Silaghi C	Supervision of screening for vector-borne pathogens
	Proofreading of manuscript
Heckel G	Proofreading of manuscript

Ulrich RG	Study design
	Writing of manuscript
	Proofreading of manuscript
Drewes S	Proofreading of manuscript

(Kathrin Jeske)

(Supervisor: Professor Dr. Rainer G. Ulrich)

Scientific articles

First authorships

Jeske K, Weber S, Pfaff F, Imholt C, Jacob J, Beer M, Ulrich RG, Hoffmann D (2019) Molecular Detection and Characterization of the First Cowpox Virus Isolate Derived from a Bank Vole. Viruses 11(11), 1075; doi: 10.3390/v11111075

Jeske K, Hiltbrunner M, Drewes S, Ryll R, Wenk M, Spakova A, Petraityte-Burneikiene R, Heckel G, Ulrich RG (2019) Field vole-associated Traemmersee hantavirus from Germany represents a novel hantavirus species. Virus genes. doi:10.1007/s11262-019-01706-7

Jeske K, Tomaso H, Imholt C, Schulz J, Beerli O, Suchomel J, Heroldova M, Jacob J, Staubach C, Ulrich RG (2019) Detection of *Francisella tularensis* in three vole species in Central Europe. Transboundary and emerging diseases 66 (2):1029-1032. doi:10.1111/tbed.13078

Drewes S*, **Jeske K***, Straková P, Balčiauskas L, Ryll R, Balčiauskienė L, Kohlhause D, Schnidrig G, Hiltbrunner M, Špakova A, Insodaitė R, Petraitytė-Burneikienė R, Heckel G, and Ulrich RG (2019) Identification of a novel hantavirus strain in the root vole (*Microtus oeconomus*) in Lithuania, Eastern Europe. Infections, Genetics and Evolutions https://doi.org/10.1016/j.meegid.2020.104520

(*contributed equally)

Co-authorships

Prkno A, Hoffmann D, Goerigk D, Kaiser M, van Maanen ACF, **Jeske K**, Jenckel M, Pfaff F (2017) Epidemiological Investigations of Four Cowpox Virus Outbreaks in Alpaca Herds, Viruses 9 (11). doi:10.3390/v9110344

Mrochen DM, Schulz D, Fischer S, **Jeske K**, El Gohary H, Reil D, Imholt C, Trube P, Suchomel J, Tricaud E, Jacob J, Heroldova M, Broker BM, Strommenger B, Walther B, Ulrich RG, Holtfreter S (2018) Wild rodents and shrews are natural hosts of *Staphylococcus aureus*. International journal of medical microbiology 308 (6):590-597. doi:10.1016/j.ijmm.2017.09.014

Obiegala A, **Jeske K**, Augustin M, Krol N, Fischer S, Mertens-Scholz K, Imholt C, Suchomel J, Heroldova M, Tomaso H, Ulrich RG, Pfeffer M (2019) Highly prevalent bartonellae and other vector-borne pathogens in small mammal species from the Czech Republic and Germany. Parasites & vectors 12 (1):332. doi:10.1186/s13071-019-3576-7

Imholt C, **Jeske K**, Jacob J, Ulrich RG (2018) Einfluss von Biodiversität auf die Prävalenz von humanpathogenen Erregern in Nagern. Journal für Kulturpflanzen 70(2): 72 (Abstract publication)

Oral presentations

Jeske K, News from the hantavirus front, *INNT-Seminar*, 29.8.2019, Insel Riems, Germany

Jeske K, Neues zu Erregern bei der Feldmaus, *Arbeitskreis Mäuse im Forst*, 27.03.2019, Münster, Germany

Jeske K, Potential effect of biodiversity on pathogen infection in the common vole *(Microtus arvalis)*, *Hausseminar*, 02.05.2019, Insel Riems, Germany

Jeske K, Effect of biodiversity on pathogen transmission and infection levels in the common vole (*Microtus arvalis*), *INNT-Seminar*, 24.01.2019, Insel Riems, Germany

Jeske K, Imholt C, Jacob J, Effect of biodiversity on pathogen transmission and infection levels in the common vole (*Microtus arvalis*), *Workshop des Netzwerkes "Nagetier-übertragene Pathogene"*, 29.11.2018, Berlin, Germany

Jeske K, Tomaso H and Ulrich R G, Pilotstudie zum Vorkommen von Francisella tularensis in Kleinsäugern, *Workshop des Netzwerkes "Nagetier-übertragene Pathogene"*, 29.11.2018, Berlin, Germany

Jeske K, Imholt C, Jacob J, Spakova A, Petraitytè-Burneikiené R and Ulrich R G, Generation of a new antigen for the detection of Tula orthohantavirus in Central Germany, 16th *Medical Biodefense Conference*, 30.10.2018, Munich, Germany

Jeske K, Imholt C, Jacob J, Ulrich R U, Kleinsäugerdiversität und Erregerprävalenz, *Mäuse im Forst*, 13.3.2018, Banzkow, Germany

Jeske K, Effect of biodiversity on pathogen transmission and infection levels in the common vole (*Microtus arvalis*), *INNT-Seminar*, 07.12.2017, Insel Riems, Germany

Jeske K, Effect of biodiversity on pathogen transmission and infection levels in the common vole (*Microtus arvalis*), *Hausseminar*, 08.06.2017, Insel Riems, Germany

Jeske K, Effect of biodiversity on pathogen transmission and infection levels in the common vole (*Microtus arvalis*), *INNT-Seminar*, 15.06.2017, Insel Riems, Germany

Jeske K, Fischer S, Imholt C, Höper D, Drewes S, Beer M, Jacob J, Ulrich R G, Krankheitserreger der Feldmaus: Eine Übersicht, *5. Workshop des Netzwerkes „Nagetier-übertragene Pathogene"*, 29.11.2016, Gießen, Germany

Posters

Jeske K, Fischer S, Emirhar D, Ruiz-Fons J F, Gonzalez-Barrio D, Strakova P, Balčiauskas L, Balčiauskienė L, Imholt C, Jacob J, Ulrich R G, Hantavirus and *Leptospira* spp. infections in European rodents, *International Symposium on Zoonoses Research*, 16.-18.10.2019, Berlin, Germany

Jeske K, Imholt, C.; Jacob, J.; Ulrich, R.G. (2019): Co-infections of Tula orthohantavirus and Leptospira spp. in common voles, *11th International Conference on Hantaviruses*, 01.-04.09.2019, Leuven, Belgium

Jeske K, Emirhar D, García J T, González-Barrio D, Pérez-Olea P, Ruiz-Fons F, Ulrich R U, *Leptospira* spp. and orthohantavirus prevalence in *Microtus* spp. voles in Spain, *Junior Scientist Zoonosis Symposium*, 20.-22.06.2019, Berlin, Germany

Jeske K, Imholt C, Jacob J, Ulrich R G, Prevalence of Tula orthohantavirus and *Leptospira* spp. in common voles, *National Symposium on Zoonoses Research*, 17.-19.10.2018, Berlin, Germany

Jeske K, Imholt C, Jacob J, Ulrich R G, Prevalence of Tula orthohantavirus and *Leptospira* spp. in common voles, *7. FLI-internes Nachwuchswissenschaftler-Symposium*, 24.-26.09.2018, Greifswald, Germany

Jeske K, Tomaso H, Imholt C, Schulz J, Beerli O, Tricaud E, Heroldova M, Staubach C, Ulrich R U, Towards a monitoring of *Francisella tularensis* in *Microtus arvalis* in Europe, *National Symposium on Zoonoses Research*, 12.10.2017, Berlin, Germany

Jeske K, Imholt C, Schmidt S, Jacob J, Heckel G, Ulrich R G, Common vole (*Microtus arvalis*) as a host for pathogens: a review, *Deutsche Gesellschaft für Säugetierkunde, 91*th *Annual Meeting*, 19.09.2017, Greifswald, Germany

Danksagung

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

(Unterschrift des Promovierenden)