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White-toothed shrews (genus *Crocidura*) - Small in size, but great in pathogen diversity

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Review 1

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Review 2

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Publication 3

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I. Introduction

The emergence of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) has demonstrated the vulnerability of global health to the impact of emerging infectious diseases (EID), of which more than 75% are of animal origin. To date, our knowledge of pathogen diversity only scratches the surface, especially those harboured by wildlife. Increasing human-wildlife interfaces due to human population growth, land use change and alterations of animal species distribution, may provoke spillover possibly resulting in the emergence of zoonotic diseases. In addition to phylogenetic relatedness, geographic proximity is a primary driver of species interactions facilitating cross-species transmission.

Globally distributed small mammals, which are the species-richest mammalian orders, inhabit a great variety of ecological niches, including human settlements, hence present ideal candidates to study pathogen dynamics such as pathogen distribution, host-switches and pathogen evolution. They have been identified as reservoir species for several highly virulent pathogens; maintaining, replicating, and shedding these agents, without suffering from disease. However, previous studies have mainly focused on bats and rodents, and the role of insectivores as reservoirs has been somewhat neglected. The recent identification of the zoonotic Langya virus (family *Paramyxoviridae*) in white-toothed shrews (*Crocidura lasiura, Crocidura shantungensis*) in China, as well as, the detection of Borna disease virus 1 (BoDV-1, family *Bornaviridae*) in bicolored white-toothed shrews (*Crocidura leucodon*) in Europe, which causes lethal encephalitis in humans and domestic animals, have drawn shrews (order Eulipotyphla) into the (scientific) spotlight.

To comprehensively understand the complexity of disease dynamics and improve human, animal, and planetary health a multidisciplinary One Health approach is required. “One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent“ (Adisasmito et al., 2022), a viewpoint which is endorsed by the world’s leading animal and human health organizations.

This thesis aims to assess the reservoir status of white-toothed shrews in Central Europe by combining ecological investigations with pathogen studies. It evaluates the (i) distribution of white-toothed shrews in Germany and their (ii) role as reservoirs for potentially zoonotic pathogens using pathogen-specific molecular detection methods and metagenomic high-throughput sequencing. (iii) Applying the holistic One Health approach, the role of white-toothed shrews, the environment and arthropods were studied alongside a sero-epidemiological investigation of BoDV-1 exposure in citizens of the municipality with the first detected paediatric BoDV-1 infection cluster in a highly BoDV-1 endemic area in Bavaria, Germany.

II. Review of Literature

2.1 White-toothed shrews

2.1.1 Order Eulipotyphla: Shrews

Shrews are small insectivorous animals belonging to the order Eulipotyphla (formerly Insectivora). They are distributed worldwide except Antarctica and Australia and occupy a wide variety of habitats. Fossil records are rare and species identification is commonly based on morphological features, despite the close resemblance between species. Currently, 448 species are known and their number is increasing with the ongoing discovery of new species (Hutterer et al., 2018; Esselstyn et al., 2021), making them the fourth largest species group within the class Mammalia. Modern phylogenies based on comparisons of mitochondrial DNA (deoxynucleic acid) and nuclear genes have revealed a very complex species diversification, which is still changing as more phylogenetic data become available, and new species are discovered, leading to reorganization of existing divisions (Hutterer et al., 2018; Esselstyn et al., 2021; Westra et al., 2022). Roughly 60 Myr (million years) ago, Soricidae (shrews) separated from Erinaceidae (hedgehogs) (Wilson and Mittermaier 2017) (**Figure 1**). Three subfamilies with 26 genera are distinguished: white-toothed shrews (Crocidurinae, 242 species, ten genera), red-toothed shrews (Soricinae, 181 species, 13 genera), and African white-toothed shrews (Myosoricinae, 25 species, three genera) (Wilson and Mittermaier 2017) (**Figure 1**)**.** The separation of Crocidurinae and Soricinae occurred roughly ~ 30-40 Myr ago. The subfamilies differ not only in morphological traits such as size, fur and tooth colour, but also in their ecology and behaviour. Among the subfamily Soricinae, *Sorex* is the most speciose genus, yet it is more uniform and less specialized than other genera (Wilson and Mittermaier 2017). Three independent lineages of Eurasian Soricinae have colonized North America during the middle Miocene (~ 13.9-12.1 Myr ago) (Dubey et al., 2007d). Soricine shrews can be found at moist habitats at higher altitudes, than crocidurine shrews and are mainly found in the Holarctic region, while extant species are absent from the African continent (Wilson and Mittermaier 2017). Crocidurine shrews are absent from the Americas, but are widespread in Eurasia and Africa, and are well adapted to tropical temperatures. Crocidurine species have evolved in Eurasia, and several colonization and recolonization events between Africa and Eurasia are described (Dubey et al., 2007d; 2008b; 2008c). Currently, the greatest species diversity of crocidurine shrews is described to be found on the African continent (Jacquet et al., 2015; Igbokwe et al., 2019).

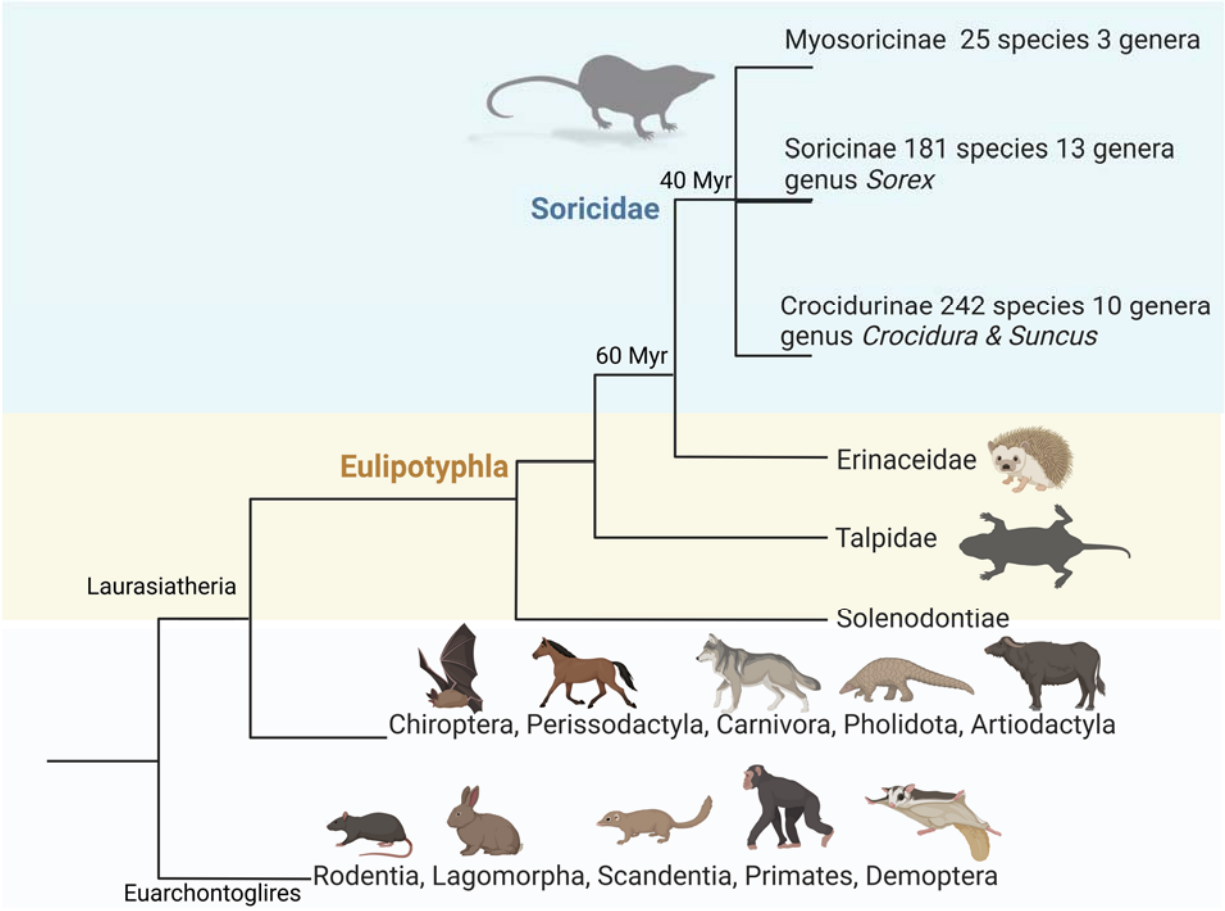

Figure 1 Dendrogram of the evolution of mammalian orders, with particular emphasis on the organization of the order Eulipotyphla. It has been estimated that the separation between Erinaceidae and Soricidae occurred ~ 60 Myr (million years) ago and the separation of the subfamilies was determined to have happened ~ 40 Myr ago (Wilson and Mittermaier 2017). Within the subfamily of white-toothed shrews, the monophyletic genus Crocidura has diversified from Eurasian Suncus more recently (~ 9.3 Myr), with the basal sister group being the Etruscan shrews (Suncus etruscus) (Biltueva et al., 2001; Dubey et al., 2007d). Created with BioRender.com agreement number: DK27LGKKSN.

2.1.2 Extant shrew species in Europe

Nine shrew species are present in Central Europe, six of which belong to the genus *Sorex* and three to the genus *Crocidura*. In regard to this thesis, more details are provided solely for the investigated species of the subfamily Crocidurinae: the bicolored white-toothed shrew (*Crocidura leucodon*), the greater white-toothed shrew (*Crocidura russula*), and the lesser white-toothed shrew (*Crocidura suaveolens*). A taxonomic overview on mentioned animal species is presented in the appendix section of this thesis. In addition, the closely related Etruscan shrew (*Suncus etruscus*) of the subfamily Crocidurinae was investigated. It is present in the wild in Mediterranean Europe and also kept in zoos, *inter alia* in Germany and Austria (Zootierliste 2023), and in laboratory settings (Geyer et al., 2022) to study its behaviour and neurosystem (Brecht et al., 2011; Anjum and Brecht 2012; Naumann et al., 2012; Ray et al., 2020; Geyer et al., 2022) (**Table 1**).

The bicolored white-toothed shrew is distributed from northern France across Germany to the Caspian Sea (**Figure 2**). Within the monophyletic clade of *C. leucodon*, two genetic subclades can be distinguished (Dubey et al., 2007c). The western clade extends from France to north-western Turkey and the eastern clade extends from Turkey to Georgia including Romania and Bulgaria. This separation was determined to have occurred during the Middle Pleistocene (~ 0.69 Myr) and was influenced by the formation of the Bosphorus Strait with increasing sea levels following the Günz glacial events (790,000-950,000 years before the present (B. P.)). However, the Bosphorus Strait seemed to be a permeable biogeographic barrier, as bidirectional exchange between the two conspecifics has been observed (Dubey et al., 2007c; Mahmoudi et al., 2019). Reports of increasing numbers of *C. leucodon* in the Czech Republic and neighbouring countries may lead to fusion of the existing distribution gap in the future (Leso et al., 2008; Andera 2010).

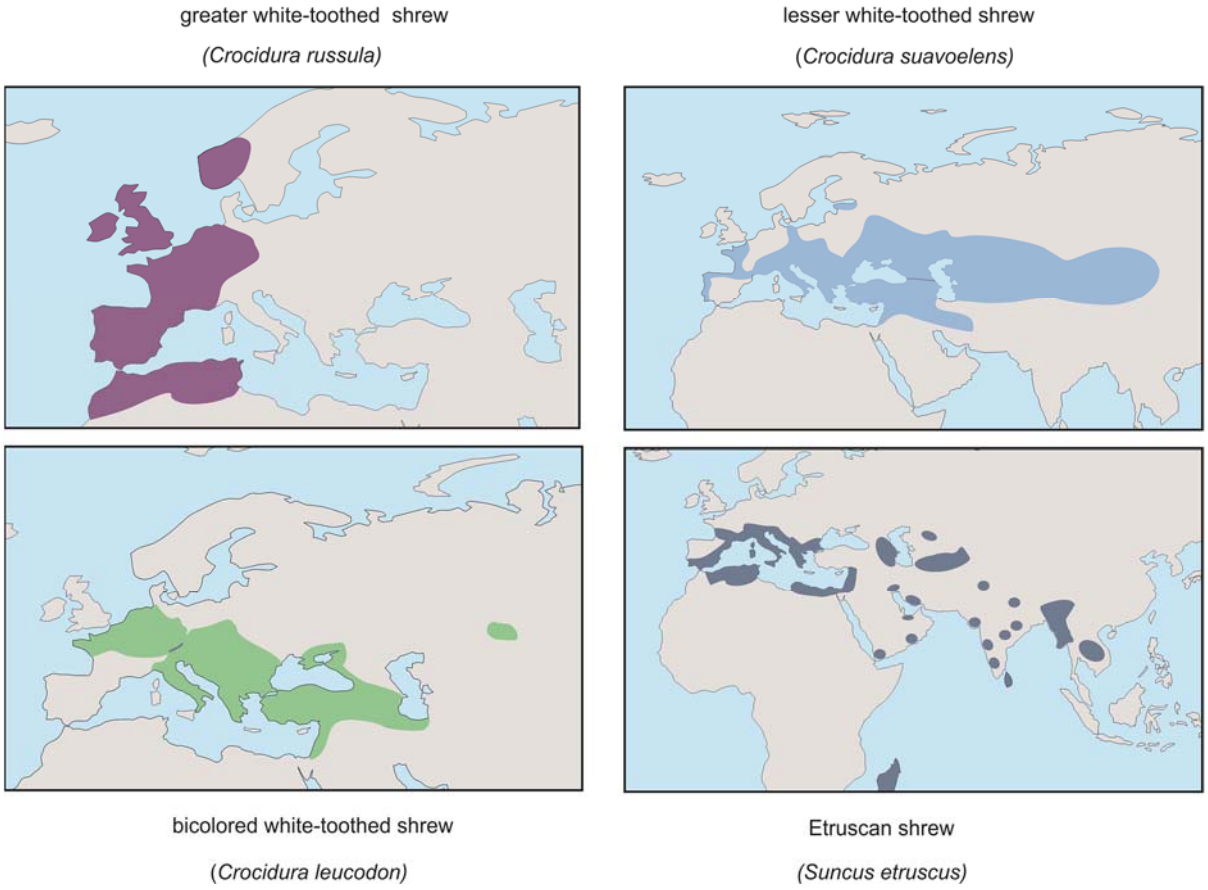

Figure 2 Current distribution of the bicolored white-toothed shrews (*Crocidura leucodon*), the greater white-toothed shrews (*Crocidura russula*), the lesser white-toothed shrews (*Crocidura suaveolens*) and the Etruscan shrews (*Suncus etruscus*) based on Wilson and Mittermaier 2017. The distribution ranges are incompletely defined and fluctuate. For *C. russula* a constant range expansion has been observed and the first descriptions from Ireland, Great Britain, Fennoscandia and the Czech Republic are considered (Tosh et al., 2008; McDevitt et al., 2014; Bond et al., 2022; van der Kooij and Nyfors 2023; Bellocq et al., 2023). Modified after Haring et al., 2024: Detection of novel orthoparamyxoviruses, orthonairoviruses and an orthohepevirus in European white-toothed shrews. MGen 10, doi.org/10.1099/mgen.0.001275.

The lesser white-toothed shrew is represented by a complex group of subspecies, for which morphological and genetic distinctions are not always congruent. Its distribution ranges from the Atlantic coast almost to the Pacific Ocean. Two separate lineages have been identified; one is found in France and northern Spain, while the other one expands from Central Europe further east to Central Asia and China (Taberlet et al., 1998; Garcia et al., 2020) (**Figure 2**). The distribution itself is patchy and not all subspecies are clearly identified and differentiated yet (Dubey et al., 2006; Dubey et al., 2008a; Gritsyshin et al., 2023; İbiş et al., 2023). The current distribution and phylogenetic studies indicate the Iberian Peninsula as possible refuge during the Last Glacial Maximum (23,000-18,000 years B. P.) (Dubey et al., 2006; Dubey et al., 2007b). Rofes et al., (2018) detected *C. suaveolens* remains at the same archaeological site as human remains, suggesting a certain degree of synanthropy since the early Chalcolithic (5,000 years B. P.). Genetic studies of different *Crocidura* species from China demonstrated a close relationship between *C. suaveolens* and the Shantung white-toothed shrew (*Crocidura shantungensis*) (Jiang and Hoffmann 2001; Bannikova et al., 2009; Lee et al., 2018; Chen et al., 2020b).

The greater white-toothed shrew originates in northern Africa and has invaded Europe about 60,000 years B. P. (Cosson et al., 2005; Brändli et al., 2005). Its range continues to expand further north and east, with recent records from Ireland (Tosh et al., 2008; McDevitt et al., 2014), Great Britain (Bond et al., 2022), Fennoscandia (van der Kooij and Nyfors 2023) and the Czech Republic (Bellocq et al., 2023). In newly colonized areas, the greater white-toothed shrew frequently outcompetes the native shrew species, which may lead to the reduction and local extinction of smaller shrew species (Neves et al., 2019), as has been observed for *C. leucodon* and *C. suaveolens* in continental Europe (Switzerland and Germany (Vogel et al., 2002; Kraft 2000; Wolf 2010)), and for the Eurasian pygmy shrew (*Sorex minutus*) in Ireland (McDevitt et al., 2014).

The genus *Suncus* contains 19 species, which are present in Eurasia and Africa. The Asian house shrew (*Suncus murinus*) is the best studied crocidurine species due to its relatively large appearance (23.5-147.3 g body weight) and commensal behaviour. It is listed at the Global Invasive Species Database (GISD) due to reaching high densities and negatively impacting local plant, invertebrate and vertebrate species, where introduced by anthropogenic movements (Global Invasive Species Database 2023). *Suncus etruscus* is next to the Hog-nosed bat (*Craseonycteris thonglongyai*) from Thailand the smallest living mammal (Bates et al., 2019). It has a scattered distribution throughout Eurasia and is mainly found in the Mediterranean area and south-eastern Europe with its most northern expansion into Switzerland (Vogel 2012; Wilson and Mittermaier 2017).

Overall, precise information on the current distribution of the different shrew species mentioned is lacking or incomplete, as they are difficult to monitor attributable to their skittish and elusive behaviour, and their small size. This is especially challenging for juveniles and the miniature species, such as *S. etruscus*, since they are too lightweight for conventional rodent traps (Fons 1973; Vogel

2012; Galán-Puchades et al., 2021). Besides the mentioned sampling bias and under-reporting, the patchy distribution may be due to natural causes such as geographical barriers (large mountain ranges, water sources), glacial era-isolation and postglacial colonization which may have led to the segregation of populations (Hewitt 2000), or due to anthropogenic movements (Schmidt 2019). Once translocated, shrews tend to quickly establish new founder populations with high genetic variance (Favre and Balloux 1997; Bouteiller and Perrin 2000; Jaquiéry et al., 2008) resulting in altered distribution ranges (Stüber 2011; McDevitt et al., 2014; Resch and Blatt 2016; Bond et al., 2022).

Shrews are rather solitary animals with strong territorial behaviour, which is more prominent in soricine than in in crocidurine shrews (Cantoni and Vogel 1989; Merten et al., 2020; Kowalski and Rychlik 2021). Detailed information on the soricine and crocidurine species in Germany and on *Suncus etruscus* are provided in **Table 1**. Reproduction strategies differ between the primarily Holarctic distributed Soricinae and Crocidurinae, with their paleotropical origin (Jeanmaire-Besançon 1988). Gestation period and litter size varies only slightly between the above-mentioned crocidurine species and is usually between 20-30 days. Litter size is on average 4-6 pubs per litter and they may have up to three litters per year. Early borne young-of the year reach sexual maturity in their first summer and start to reproduce (precocious maturity) (Jeanmaire-Besançon 1988). Life expectancy ranges from one to three years in the wild and in captivity, respectively (Geyer et al., 2022). Most wild individuals survive only one winter (fist winter survival ~ 50% (Bouteiller and Perrin 2000), second winter survival < 95%) (Henttonen et al., 1989). Female monogamy has been observed in the crocidurine species and nests are shared only with close relatives (Cantoni and Vogel 1989; Bouteiller and Perrin 2000). They are crepuscular or even nocturnal. Energy expenditure is very high, resulting in a need for constant foraging and feeding. They feed on beetles, arthropods, worms and molluscs (Wolf 2011). In the case of the highly abundant common shrew (*Sorex araneus*) this results in significant biomass cycling as an important ecosystem service (Wilson and Mittermaier 2017). The semi-aquatic *Neomys* spp. may be used as indicator species of healthy water systems (Briner et al., 2021).

For the reduction of energy demands and to cope with temperate climate torpor is induced (Nagel 1977) and winter aggregation has been described for the more social crocidurine shrews (Cantoni and Vogel 1989; Kraft 2008). *Sorex araneus* has developed an alternative wintering strategy, the so called ‘Dehnel’s Phenomenon’. This is the size and mass reduction of the skull, the brain, and other internal organs, resulting in reduced energy demands for thermoregulation, albeit its larger body surface (Schaeffer et al., 2020).

Table 1 Biological and ecological information on shrew species present in Germany and on the Etruscan shrew (*Suncus etruscus*) obtained from Wilson and Mittermaier 2017.

Soricinae – genus *Sorex*

Sorex araneus, KS22/2721, taken by Viola C. Haring

	***Sorex araneus* Linnaeus, 1758** **common shrew** **Waldspitzmaus**	***Sorex minutus* Linnaeus, 1766** **Eurasian pygmy shrew** **Zwergspitzmaus**	***Sorex coronatus* Millet, 1828** **crowned shrew** **Schabrackenspitzmaus**	***Sorex alpinus* Schinz, 1837** **Alpine shrew** **Alpenspitzmaus**
Information on body size, tail length, body weight of adults	Head-body length: 56-82 mm, tail length: 37-52 mm, weight: 5-14.5 g	Head-body length: 40-64 mm, tail length: 33-45 mm, weight: 2.6-7 g	Head-body length: 68-80 mm, tail length: 37-46 mm, weight: 6.5-11.8 g	Head-body length: 62-77 mm, tail length: 60-75 mm, weight: 5.5-11.5 g
Habitat	Forests with predominance of deciduous species and grass covers, temporarily in grassy meadows	Forest-tundra, steppe; avoids coniferous forests	Highland coniferous and plain-broad-leaved forests	Highlands with sparse trees and shrubs
Distribution	Great Britain - continental Europe - central Siberia	Europe to Siberia	North Spain – France – Switzerland - Germany	Alpine area - southeast France - north Albania
Status and Conservation	Least Concern	Least Concern	Least Concern	Near Threatened
Additional Information	Most numerous and eurytopic, significant role in biomass circulation	Rarely dominant when sympatric with other species	Abundant in all regions	European endemic and relict species

Table to be continued.

Soricinae – genus *Neomys*

Neomys anomalus KS22/2722, taken by Viola C. Haring

	***Neomys anomalus* Cabrera, 1907** **Mediterranean water shrew** **Sumpfspitzmaus**	***Neomys fodiens* (Pennant, 1771)** **Eurasian water shrew** **Wasserspitzmaus**
Information on body size, tail length, body weight of adults	Head-body length: 68-85 mm, tail length: 46-56 mm, weight: 9.5-13.5 g	Head-body length: 75-103 mm, tail length: 58-73 mm, weight: 8.5-25 g
Habitat	Floodplain habitats, banks of small rivers, irrigated gardens, semi- aquatic	Forest zones, forest-steppe/tundra, neighbouring subzones, avoids extended wood area, high grass, near water, also gardens
Distribution	Iberian Peninsula - west Russia	Atlantic Coast - Altai steppes - Pacific coast
Status and Conservation	Least Concern	Least Concern
Additional Information	Possible relict species, paralytic saliva, social, live in groups	Solitary, paralytic saliva, may survive second winter, indicator species of aquatic systems (Briner et al., 2021)

Table to be continued.

Crocidurinae – genus *Crocidura* and genus *Suncus*

Crocidura leucodon, taken by Henning Vierhaus

	Crocidura leucodon **(Hermann, 1780)** **bicolored white-toothed shrew** **Feldspitzmaus**	***Crocidura suaveolens*** **(Pallas, 1811)** **lesser white-toothed shrew** **Gartenspitzmaus**	***Crocidura russula*** **(Herrmann, 1780)** **greater white-toothed shrew** **Hausspitzmaus**	***Suncus etruscus*** **(Savi, 1822)** **Etruscan shrew** **Etrusker Spitzmaus/** **Wimperspitzmaus**
Information on body size, tail length, body weight of adults	Head-body length: 59-72 mm, tail length: 31-41 mm, weight: 5.9-11.1 g	Head-body length: 47-80 mm, tail length: 25-40 mm, weight: 6.5-9.4 g	Head-body length: 44-86 mm, tail length: 24-47 mm, weight: 5-16 g	Head-body length: 33-50 mm, tail length: 21-30 mm, weight: 1.2-2.7 g
Habitat	Various open biotopes, wet and forest habitats are avoided	Desert, steppe, forest zones	Wide variety of habitats, shrubs, open habitats	Lowland, lower belts of mountain ranges, xeromorphic shrubs
Distribution	Northwest-France – South/Central Europe to the Caspian Sea	West France - Germany - Caspian Sea until Central China	North Africa - Iberian Peninsula - Germany, commensal in northern Europe	Mediterranean area, until Vietnam, including Madagascar – but very scattered, many subspecies
Status and Conservation	Least Concern	Least Concern	Least Concern	Least Concern
Additional Information	Bicolored fur	Smallest crocidurine species present in Germany	Quickly adapt to habitat loss, predominate over other (crocidurine) species	Smallest living mammal

2.2 Pathogen dynamics

2.2.1 What defines a reservoir?

To comprehensively grasp the specifics of a disease, one must familiarise oneself with the pathogen-reservoir-host system. However, what constitutes a reservoir?

The term ‘reservoir’ is loosely defined as an ‘ecological system in which an infectious agent survives indefinitely’ (Ashford 1997), but precise definitions are controversially discussed (Haydon et al., 2002; Ashford 2003; Viana et al., 2014). In addition to biotic systems (Eukaryota and Prokaryota), also the environment (e.g. water, soil) may act as a reservoir. The following characteristics are applied for the definition of reservoirs used for this study (Haydon et al., 2002; Power and Mitchell 2004; Hallmaier-Wacker et al., 2017):

i) the reservoir harbours and maintains the pathogen lifelong,

ii) rarely shows clinicals signs,

iii) the pathogen is transmitted to other individuals.

Transmission occurs through direct or indirect contact routes (**Figure 3**). If a pathogen`s infection cycle involves a reservoir, in-depth knowledge of the target (negatively affected population)-reservoir system is required to establish effective preventive measures to reduce infection. Limited information on the reservoir only allows infection prevention and management via target control efforts by e. g. vaccination of the target population. Expanding knowledge of the reservoir in contrary, enables the establishment of blocking tactics e.g. direct blocking of transmission between the reservoir and the target, or even direct control of the reservoir through means such as culling, vaccination, or treatment programs (Haydon et al., 2002).

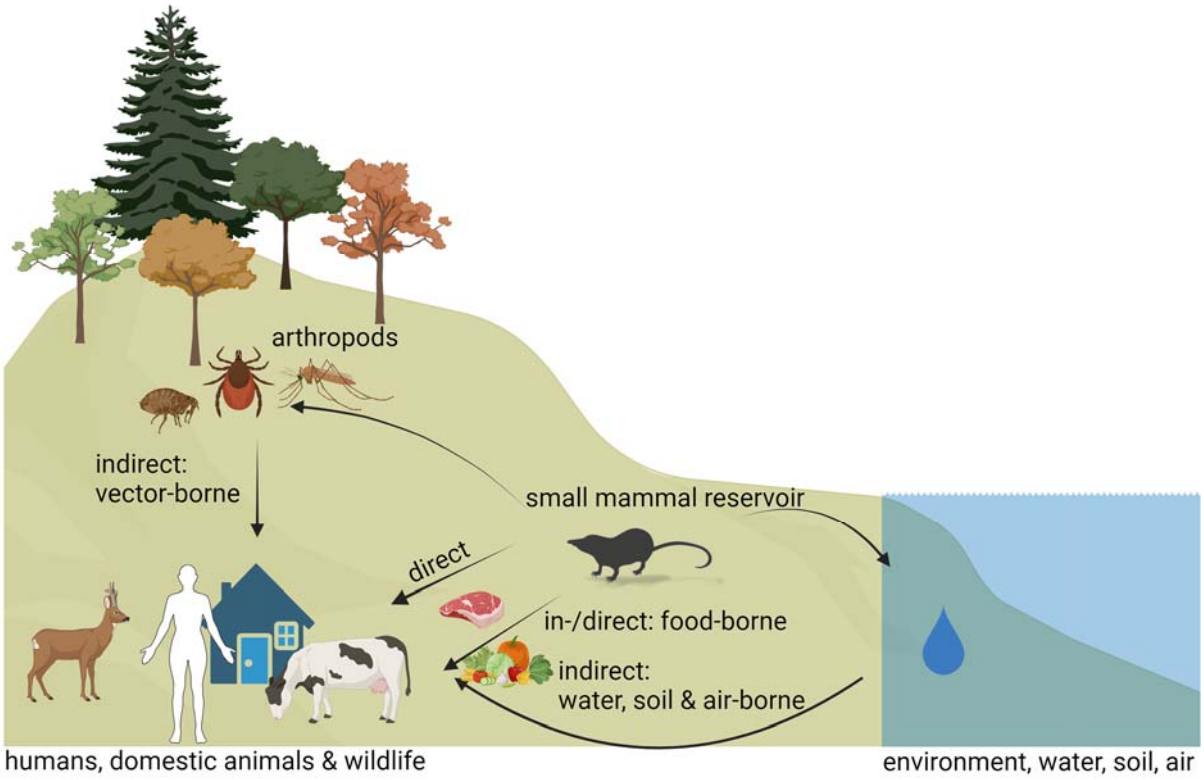

Figure 3 Transmission routes of pathogens. Pathogens are transmitted via different routes between a reservoir (i. e. small mammal species) and a new host (humans, domestic animals, wildlife). Direct transmission routes are contact, or consumption of animal-based food products; and indirect transmission routes are arthropod-borne, air-borne, water-borne, consumption of contaminated (plant-based) food, soil-borne. Created with BioRender.com agreement number: OF25WQ6LT7.

Managing target-reservoir systems relies on the awareness and comprehensive understanding of the pathogens present. Hence, knowledge on pathogen diversity in wildlife is essential to monitor 'emerging infectious diseases' (EIDs) (Jones et al., 2008; Dharmarajan et al., 2022). It is crucial to prepare effective countermeasures as EIDs may have dramatic consequences. EIDs may possess high pathogenicity, which describes the ability of a pathogen to induce mortality and fitness loss, and high virulence, which is the degree of the pathogenicity and subsequently induced harm (Casadevall and Pirofski 1999) as seen for Ebola virus (EBOV (Baize et al., 2014). They may have the potential to cause pandemics as seen with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 (Hiscott et al., 2020) and human immunodeficiency virus 1 (HIV-1 (Hahn et al., 2000; Simon et al., 2006)), or have high economic impacts as observed for avian and swine influenza (Neumann et al., 2009; Martini et al., 2019) and African swine fever virus (ASFV (Berthe 2020)). Of all emerging human diseases, 75% are of animal origin, either wildlife or livestock (Jones et al., 2008; WOAH 2023a; Carlson et al., 2019). Indeed, even for well-known pathogens like rubella virus (RuV), which was considered to be exclusive to humans (Wolfe et al., 2007; Woolhouse et al., 2013), phylogenetic relatives were discovered within the animal kingdom, indicating a potential animal origin (Bennett et al., 2020). Of note, a taxonomic overview on mentioned virus species is presented in the appendix section of this thesis. RNA

(ribonucleic acid) viruses are more likely to emerge than DNA viruses, mainly due to their error-prone RNA-directed RNA polymerase (RdRp), which leads to a higher frequency of mutations (Simpson et al., 2020). This, in turn may trigger evasion of host response, spillover and adaptation to new hosts (Cleaveland et al., 2001).

2.2.2 Pathogen evolution and disease dynamics

Host range is a significant factor in categorizing and assessing novel pathogens. Multi-host pathogens, that affect human, domestic animals, and wildlife, have a profound impact on both human and animal health and welfare, also considering socio-economic consequences (Cleaveland et al., 2001; Smith et al., 2019). As pathogens exploit niches and adapt to new hosts, their emergence in new species could also be considered a logical consequence of pathogen ecology and evolution (Karesh et al., 2012). One of the major opportunities for the emergence of zoonotic diseases occurred with the domestication of animals, which has led to the evolution of the ancestors of measles virus (MeV) and smallpox viruses (VARV) (Wolfe et al., 2007; Düx et al., 2020). Domestic animals play a crucial role in virus sharing among multiple mammalian hosts, including humans due to their high density and close proximity to humans (Johnson et al., 2020; Dharmarajan et al., 2022), which is particularly evident for DNA viruses (Wells et al., 2020). However, different wildlife species play a crucial role in the spread of RNA viruses such as bats, ~ 85% of all bat associated viral sequences available on GenBank have an RNA genome (van Brussel and Holmes 2022), and carnivores (Cleaveland et al., 2001; Luis et al., 2013). Host-specificity is lower in RNA viruses compared to DNA viruses, facilitating host-switches across ecological niches (Wells et al., 2020). Although comparisons between domestic animals and wildlife may be fraudulent due to sampling biases such as accessibility of samples, sample type and funding (EFSA-ALPHA UNIT 2014; Olival et al., 2017; Schilling et al., 2022). Described viral richness is positively associated with sampling efforts and sympatry of species allowing interspecies transmission (Luis et al., 2013). Surveillance of species-specific pathogens in wildlife is essential to monitor for host-switches and EIDs (Rothenburg and Brennan 2020). In case of a spillover event, the virus jumps from one species (reservoir) to another species (spillover host) (Plowright et al., 2017) (**Figure 4**). The interaction between the new host species and the pathogen may lead to different outcomes (Rothenburg and Brennan 2020). Pathogenicity and virulence may be low or even absent in the reservoir species, but may be different in another host species, where it could be highly virulent, highly lethal or rapidly onward transmitted (Casadevall and Pirofski 1999). Infected individuals that either succumb to death, achieve asymptomatic persistence, or clear the pathogen, do not establish stable onward transmission. Therefore, they are defined as (accidental) dead-end hosts. If the new host species is a suitable environment for the pathogen or the pathogen adapts to the new host, onward transmission may be possible and the new host is defined as amplifying or intermediate host (Markotter et al., 2020).

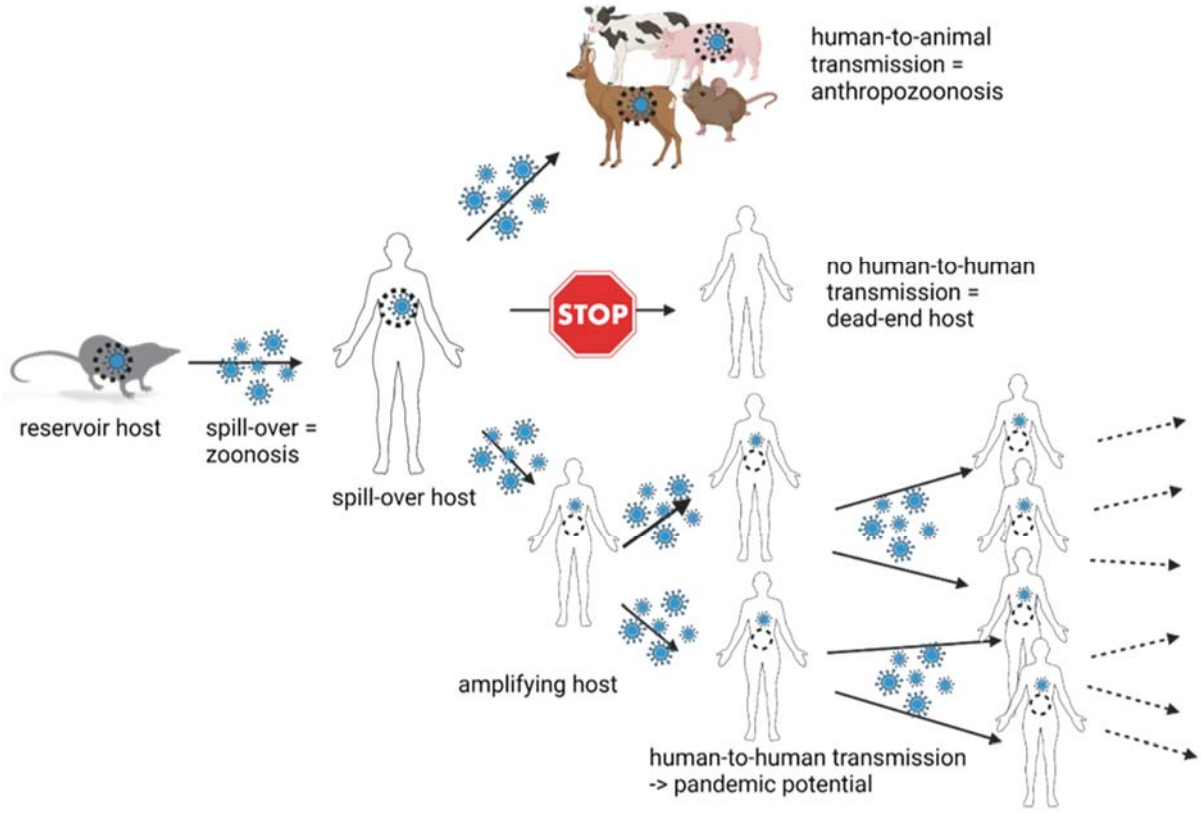

Figure 4 Evolution of potentially zoonotic 'Emerging infectious diseases' (EIDs). In the case of a spillover event from an animal (reservoir) to humans, a new zoonosis may establish. The pathogen may be further transmitted to animals, being than defined as anthropozoonosis, or transmitted to other humans. Replication and transmission take place in this new amplifying host. Infection of a new species can also lead to a dead-end without onward transmission, as the new host clears the infection, dies or remains persistently, but non-infectiously infected. Created with BioRender.com agreement number: PA25S5SLGE.

The greatest risk for the evolution of pandemics is associated with pathogens that demonstrate easy human-to-human transmission (Geoghegan et al., 2016; Morse et al., 2012; Dharmarajan et al., 2022), as exemplified by SARS-CoV-2 (Zhou et al., 2020) and influenza viruses (Walther and Ewald 2004; Richard et al., 2017; Martini et al., 2019). Human-to-human transmission is more easily achieved by viruses that are multi-segmented, replicate in the cytoplasm, and are transmitted via aerosols (Pulliam and Dushoff 2009; Luis et al., 2013). Co-divergence, the simultaneous evolution of host and virus, is often observed in DNA viruses such as polyomaviruses (Gedvilaite et al., 2017; Ehlers et al., 2019; Moens et al., 2017), and may lead to pathogen-host adaptation and absence of host pathogenicity. This has also been observed in bacteria, i.e. for the *Leptospira* genus, which has co-evolved with its maintenance hosts (Lei and Olival 2014). However, there are also exceptions, such as pox viruses, which have maintained high virulence despite a long common history (Haller et al., 2014). Another exception are lyssaviruses (family *Rhabdoviridae)*, the causative agents of rabies, which induce disease in its reservoir resulting in behavioural changes enabling onward transmission through direct contact, mainly biting (Fisher et al., 2018). Although, its natural reservoirs are bats, rabies virus (RABV) has

adapted to dogs and other carnivores. More than 99% of human infections result from dog mediated rabies (WOAH 2023b). Behavioural differences between animals and humans require the development of new strategies for effective human-to-human transmission (Richard et al., 2017). However, the globalized and industrialized world has created artificial opportunities for transmission through means such as blood transfusions (hepatitis C (HCV) and West Nile virus (WNV)), intravenous drug application (HIV-1), vaccine production (Simian virus 40 (SV40)), and international travel (SARS-CoV-2, cholera) (Pealer et al., 2003; Wolfe et al., 2007)). Interspecies transmission is influenced by the pathogen's host range, co-existence of host species, and phylogenetic relatedness of species as they may share similar characteristics (e.g. molecular and immunological factors, and/or ecological similarities) (Morse et al., 2012; Stephens et al., 2019; Olival et al., 2017; Dallas et al., 2019). Additional factors that present opportunities for pathogen transmission and spillover are predator-prey interactions, as pathogens may accumulate along the food chain (Lafferty et al., 2006; Malmberg et al., 2021). This phenomenon has been observed for apex predators, as exemplified by the dramatic outbreak of canine distemper virus (CDV) among Serengeti lions (*Panthera leo*) (Cleaveland et al., 2000), as well as, highlighted by human consumption of (undercooked) meat, which may carry *Salmonella* spp. and *Campylobacter jejuni* in poultry (Fegan et al., 2022), variant Creutzfeld-Jakob disease (vCJD) from consumption of beef (Beekes 2010), and human hepatitis E virus (HEV) in pork (WHO 2023). In addition to zoonoses, where humans are infected with a pathogen that originated in animals, anthropozoonoses refer to cases where pathogens are transmitted from humans to animals. Anthropozoonoses pose a major threat for the conservation of endangered species, such as our closest relatives, the non-human primates (Köndgen et al., 2017), for other wildlife (Dobson and Foufopoulos 2001; Hale et al., 2022), and for livestock and pets (Račnik et al., 2021; da Silva et al., 2021) (**Figure 4**).

2.3 Metagenomics and the discovery of viruses

2.3.1 Microbiome

'Microbiome is the community of microorganisms living together in any given habitat' and was defined for the first time by Whipps et al., in 1988. One of the most cited definitions of the microbiome in an ecological context was proposed by Lederberg and McCray (2001) as 'community of commensal, symbiotic, and pathogenic microorganisms within a body space or environment'. Recently, a panel of specialists in microbiome research discussed amendments to this initial definition by Whipps et al., (1988), addressing the complexity of the interactions influencing the microbiome (Berg et al., 2020). Of great controversy is the definition of microbiota, which currently only includes living members, and excludes phages, plasmids, mobile genetic elements, and eukaryotic viruses. Eukaryotic viruses are the

key study objectives of this thesis. However, these non-living components still belong to the microbiome as 'theatre of activity', as already defined by Whipps et al., (1988).

2.3.2 High-throughput sequencing, metagenome, virome and virus discovery

It is possible to determine an organism's 'virome' by means of unbiased, hypothesis-free high-throughput sequencing of the metagenome (mHTS), which represents all nucleic acids present at the time point of assessment. This provides insight into the viral diversity, abundance and structure of the so-called virosphere (Zhang et al., 2019). It has been successfully employed for virus discovery in clinical (Hoffmann et al., 2015; Höper et al., 2016; Wilson et al., 2019; Bennett et al., 2020; Zhang et al., 2022b) and non-clinical context (Drewes et al., 2017; Cholleti et al., 2022).

After the pioneering development of the first-generation sequencing (FGS) synchronously by Maxam and Gilbert (1977) and Sanger et al., (1977), which allowed the sequencing of clonal DNA populations, the latter also known as Sanger sequencing applying the dideoxyribonucleoside chain-termination method, has prevailed (Sanger et al., 1977; Liu et al., 2012). A new era of whole-genome sequencing (WGS) has commenced with the objective of determining the entire nucleic acid sequences of (viral) genomes. Advancements of the second-generation sequencing (SGS) have facilitated this endeavour (Deurenberg et al., 2017), applying high-throughput sequencing (HTS) by parallelizing numerous reactions also leading to reduced expenses (Lipkin 2013; NIH 2021).

A common SGS-system is Illumina Inc.'s Sequencing-by-Synthesis, which has four basic steps (Illumina, Inc. 2017).

i. Library preparation: the cDNA (copy DNA) samples are fragmented, labelled with 5'- and 3'-adapters, PCR (polymerase chain reaction) amplified and loaded onto a flow cell
ii. Clusters are generated by amplification of DNA fragments
iii. Sequencing is based on the detection of single nucleotides
iv. Data analysis: The paired sequence reads (contigs) need to be trimmed, filtered and corrected, before they can be aligned either to a reference sequence or *de novo* assembled

Nowadays, third-generation sequencing (TGS), such as Oxford Nanopore Technologies (ONT), has evolved (van Dijk et al., 2018). These long-read technologies do not require timely preparation of amplification libraries, drastically reducing time and cost compared to SGS. They are extremely useful for rapid sequencing in outbreak scenarios (King et al., 2020) and low-resource settings (Quick et al., 2016), but are limited by their high sequencing error rate, making e.g. the SGS the better choice for unbiased metagenomic approaches (Höper et al., 2016; López-Labrador et al., 2021).

Widely used generic diagnostic methods, such as pan-PCR protocols, which are based on conserved genome sequences, have been successfully applied for the detection of (novel) pathogens in rodents and other small mammals (Klempa et al., 2007; Drexler et al., 2012; Drexler et al., 2015; Rasche et al.,

2019; Johne et al., 2019) (**Table 2** and **Table 3**), but are limited in deciphering the entire virome (Zhang et al., 2019). Overall, appropriate sample selection is a key factor for the successful detection and characterization of a pathogen (Höper et al., 2017). In case of limited sample material, such as cerebrospinal fluid (CSF) in encephalitis cases, mHTS may be the appropriate choice (Brown et al., 2018; Wilson et al., 2019; Hong et al., 2020). In case of a sick or deceased animal, a thorough patho-(histo)logical examination can provide first indications as to the possible cause of death, and further analysis may be initiated (Krogstad and Dixon 2003), which enhances the detection of the causative agent (Hoffmann et al., 2012; Abendroth et al., 2017; Forth et al., 2018; Fereidouni et al., 2019; Weissenböck et al., 2022). Unfortunately, in-depth pathological examinations in small mammals are difficult due to their high metabolism and rapid onset of autolysis (Krogstad and Dixon 2003). Nevertheless, they might be obsolete for studies on reservoir species as detectable pathogenesis is usually not induced by the carried pathogens.

2.4 White-toothed shrews as reservoirs

Small mammals act as a link for pathogen transmission among humans, domestic animals, wildlife and arthropod-vectors like ticks, mites, and fleas (Paziewska et al., 2010; Karbowiak et al., 2016; Defaye et al., 2022) (**Figure 3**). Ecke et al., (2022) have described a positive correlation between the number of (zoonotic) pathogens per rodent species and their synanthropic behaviour and human exploitation. Small mammals are a species-rich group mainly represented by the three orders: Rodentia (~ 2,277 species, 481 genera), Chiroptera (~ 1,116 species, 45 genera), and Eulipotyphla (~ 452 species, 55 genera) (Wilson and Reeder 2005; Wilson and Mittermaier 2017). As many of these species have not been sufficiently studied in regards to their ecological, biological and immunological properties, caution must be taken when drawing conclusions across species. Han et al., (2015) proposed the term ‘hyperreservoir’ for species that carry two or more zoonotic agents. They have established a prediction model identifying rodent reservoir and hyperreservoir species based on intrinsic traits, which are less susceptible to bias. In total 86 factors, such as early sexual maturity, large litter size, short gestation period, and postnatal growth rate, were considered and led to the identification of geographical reservoir hot spots and additional species which may guide future research focus. High abundance, species richness, and adaptability to changing environments, have been postulated that render small mammals as competent reservoirs (Drexler et al., 2012; Luis et al., 2013)

Previous reservoir studies have focused mainly on rodents and bats (Wang et al., 2011; Luis et al., 2013; Hayman 2016; Nieto-Rabiela et al., 2019; Wang et al., 2023a; Zhou et al., 2020), with the successful identification of reservoir species for highly virulent pathogens (Jonsson et al., 2010; Milholland et al., 2018): Lassa virus (LASV) in Natal mastomys (*Mastomys natalensis*) (Happi et al., 2022), lymphocytic choriomeningitis virus (LCMV) in house mice (*Mus musculus*) (CDC-DHCPP 2014),

severe respiratory syndrome coronavirus (SARS-CoV) in bats (Li et al., 2005), Hendra virus (HeV) and Nipah viruses (NiV) in fruit bats (Field et al., 2007; Chua et al., 2002). Shrews and other insectivores, on the other hand, have been almost overlooked. However, the recent discovery of highly zoonotic pathogens in shrews, namely Langya virus (LayV) in China (Zhang et al., 2022b) and Borna disease virus 1 (BoDV1) in Germany (Dürrwald et al., 2014b; Niller et al., 2020; Schlottau et al., 2018; Korn et al., 2018), have brought them into the scientific spotlight (**Table 2** and **3**). Interactions between pathogens and the shrew microbiota, as well as pathogen diversity, patterns of pathogen co-infection and competition dynamics remain largely unexplored.

2.4.1 Pathogens in selected shrew species - Viruses

A literature review of selected viruses, bacteria, and endoparasites in shrews is presented in **Table 2** and **Table 3**, providing an overview of the status quo of pathogen investigations in shrews. However, it does not presume to be complete. To determine the true reservoir status of shrews for the chosen pathogens, it is necessary among others to determine host-specificity by investigating sympatric species. In addition, systematic longitudinal studies are required to acquire comprehensive data on the geographical distribution and persistence of a pathogen in the small mammal community.

Among the subfamily Crocidurinae*, Crocidura shantungensis*, Ussuri white-toothed shrew (*Crocidura lasiura*) and *Suncus murinus* from Asia, and the African giant shrew (*Crocidura olivieri*) from Africa present to be the most extensively studied species. Previous research on shrews from Europe has mainly centred around the soricine subfamily, with its most abundant representative, the *Sorex araneus*. Several viruses have been detected in a single shrew species, and although the properties of these pathogens are not yet fully determined, it may be advised to evaluate the (hyper) reservoir status of shrews using a model similar to that of Han et al., (2015).

Table 2 Current knowledge on viruses in white-toothed shrews and selected red-toothed shrews. Publicly accessible literature was searched via 'PubMed' until July 2023 utilizing the keywords '*Crocidura'*, 'white-toothed shrews', 'Soricidae', '*Suncus'*, or 'shrews' in combination with 'virus', 'metagenome', or 'virome'. Only those studies which used direct virus detection with accessible sequences were included. Those based solely on serology were excluded. The classification of the viruses adhered to the current standards of the International Committee on Taxonomy of Viruses (ICTV), whilst descriptions of virus species followed those proposed in their respective publication.

Genome organization	Order	Family	Virus species	Detected in	Country of description	Method	Tissue	Reference
-ssRNA[a]	*Bunyavirales*	*Hantaviridae*	Azagny virus	*Crocidura obscurior*	Côte d'Ivoire	RT-PCR + sequencing	lung tissue	Kang et al., 2011a
			Tanganya virus	*Crocidura theresae* *Crocidura somalica*	Guinea Somalia	RT-PCR + sequencing	no information various organs	Klempa et al., 2007; Omoga et al., 2023
			Bowé virus	*Crocidura douceti*	Guinea	RT-PCR + sequencing + SGS	intercostal muscle	Gu et al., 2013b
			Thottapalayam virus	*Suncus murinus*	China Nepal India	RT-PCR + sequencing	lung	Song et al., 2007; Lin et al., 2014; Kang et al., 2011b; Guo et al., 2011; Wang et al., 2017a; Carey et al., 1971
			Imjin virus	*Crocidura lasiura* *Crocidura shantungensis*	Korea China	RT-PCR + sequencing	lung	Gu et al., 2011; Song et al., 2009; Sun et al., 2017
			Boginia virus	*Neomys fodiens*	Finland Poland	RT-PCR + sequencing	lung	Ling et al., 2014; Gu et al., 2013a
			Seewis virus	*Sorex araneus* *Sorex minutus* *Sorex tundrensis* *Sorex daphaenodon*	Germany Finland Russia	RT-PCR + sequencing	lung	Ling et al., 2014; Schlegel et al., 2012; Yashina et al., 2023
			Lena river virus	*Sorex caecutiens* *Sorex minutissimus*	Russia	RT-PCR + sequencing	lung	Yashina et al., 2021
			Altai virus	*Sorex minutus*	Russia	RT-PCR + sequencing	lung	Yashina et al., 2021
			Yakeshi virus	*Sorex unguiculatus*	Russia	RT-PCR + sequencing	lung	Yashina et al., 2023,
			Asikkala virus	*Sorex minutus*	Czech Republic, Germany, Finland	RT-PCR + sequencing	lung	Radosa et al., 2013; Ling et al., 2014

Table to be continued.

Genome organization	Order	Family	Virus species	Detected in	Country of description	Method	Tissue	Reference
			Jeju virus	*Crocidura shantungensis* *Crocidura lasiura*	Republic of Korea	RT-PCR + sequencing	lung	Lee et al., 2020; Seo et al., 2022Arai et al., 2012
			Jemez Springs virus	*Sorex monticolus*	USA	RT-PCR + sequencing	lung	Arai et al., 2008
			Ash River virus	*Sorex cinereus*	USA	RT-PCR + sequencing	lung	Arai et al., 2008
			Kenkeme virus	*Sorex roboratus*	China Russia	RT-PCR + sequencing	not specified	Kang et al., 2010; Wang et al., 2014
		Arenaviridae	Wenzhou virus	*Suncus murinus*	China	RT-PCR + sequencing	several organs	Li et al., 2015
			Lassa virus	*Crocidura* spp.	Nigeria	RT-qPCR	not specified	Happi et al., 2022
		Nairoviridae	Thiafora virus	*Crocidura* spp.	Senegal	TEM	not specified	Zeller et al., 1989; Walker et al., 2015
			Erve virus	*Crocidura russula*	France	TEM, inoculation of mice	spleen, kidney	Chastel et al., 1989; Dilcher et al., 2012
			Lamusara virus	*Crocidura goliath*	Gabon	RT-PCR + sequencing	kidney	Ozeki et al., 2022
			Lamgora virus	*Crocidura goliath*	Gabon	RT-PCR + sequencing	kidney	Ozeki et al., 2022
			Cencurut virus	*Suncus murinus*	Singapore	SGS + RT-qPCR	lung, spleen, kidney	Low et al., 2023
	Mononegavirales	*Paramyxoviridae*	Langya virus	*Crocidura lasiura* *Crocidura shantungensis*	China	RT-qPCR	tissue	Zhang et al., 2022b
			Melian virus	*Crocidura grandiceps*	Guinea	RT-PCR + TGS	kidney	Vanmechelen et al., 2022
			Denwin virus	*Crocidura russula*	Belgium	RT-PCR + TGS	kidney	Vanmechelen et al., 2022
			Gamak virus	*Crocidura lasiura*	Republic of Korea	SGS + RT-PCR	kidney	Lee et al., 2021

Table to be continued.

Genome organization	Order	Family	Virus species	Detected in	Country of description	Method	Tissue	Reference
			Daeryong virus	*Crocidura shantungensis*	Republic of Korea	SGS + RT-PCR	kidney	Lee et al., 2021
			henipa-related virus	*Crocidura hirta*	Zambia	RT-PCR + sequencing	kidney	Sasaki et al., 2014
			Beilong virus	*Crocidura lasiura* *Crocidura shantungensis* *Suncus murinus*	China	RT-PCR + sequencing	intestine content	Chen et al., 2020a
		Rhabdoviridae	Mokola lyssavirus	*Crocidura flavescens*	Nigeria, Cameroon	mice inoculation	non-neuronal tissue	McMahon et al., 2021; Familusi et al., 1972; Causey et al., 1969; Shope et al., 1970
		Bornaviridae	Borna disease virus 1	*Crocidura leucodon*	Germany, Switzerland, Austria	RT-qPCR + sequencing	brain	Hilbe et al., 2006; Dürrwald et al., 2014b
+ssRNA[b]	*Stellavirales*	*Astroviridae*	astrovirus	*Crocidura attenuata*	China	RT-PCR + sequencing	rectal swab	Hu et al., 2014
	Nidovirales	*Coronaviridae*	shrew CoV cluster 19 (Q)/Alpha	*Crocidura goliath*	Cameroon	RT-PCR + sequencing	rectal swab	Ntumvi et al., 2022
			Wencheng Sm shrew CoV	*Suncus murinus*	China	RT-PCR + sequencing	faecal sample	Wang et al., 2017b
			alphacoronavirus	*Sorex araneus*	Great Britain	RT-PCR + sequencing	liver	Tsoleridis et al., 2016
		Arteriviridae	*Olivier's shrew virus 1 "Crocartervirus"*	*Crocidura olivieri*	Guinea	TGS +SGS	serum	Vanmechelen et al., 2018
	Hepelivirales	*Hepeviridae*	rat hepatitis E virus	*Suncus murinus*	China	RT-PCR + sequencing	serum	Guan et al., 2013; He et al., 2018; Wang et al., 2017a
	Amarillovirales	*Flaviviridae*	hepacivirus	*Suncus murinus*	China	RT-PCR + sequencing	liver, lung, intestine	Guo et al., 2019
			Usutu virus	*Crocidura* spp.	Senegal	RT-PCR + sequencing	tissue	Diagne et al., 2019
	Picornavirales	*Picornaviridae*	hepatitis A virus	*Sorex araneus*	Germany	RT-PCR + sequencing	tissue	Drexler et al., 2015

Table to be continued.

Genome organization	Order	Family	Virus species	Detected in	Country of description	Method	Tissue	Reference
dsRNA[c]	*Reovirales*	*Spinareoviridae*	orthoreovirus	*Crocidura hirta*	Zambia	RT-PCR + sequencing	intestinal content	Harima et al., 2020
		Sedoreoviridae	rotavirus A	*Suncus murinus* *Sorex araneus*	Bangladesh Germany	RT-qPCR + sequencing SGS + RT-PCR	rectal swabs intestine	Islam et al., 2023a Johne et al., 2019
dsDNA[d]	Herpesvirales	Orthoherpesviridae	shrew herpesviruses	*Crocidura olivieri*	Kenya	PCR + sequencing	lung, kidney, liver	Ochola et al., 2022
			Brest herpesvirus	*Crocidura russula*	France	virus isolation, TEM	not specified.	Chastel et al., 1994; Hughes et al., 2010
			herpesvirus	*Crocidura* spp.	DRC	PCR + sequencing	liver, spleen	Ntumvi et al., 2018
			gammaherpesvirus	*Suncus murinus*	China	PCR + sequencing	rectal swabs	Zheng et al., 2016
	Blubervirales	*Hepadnaviridae*	hepatitis B virus	*Sorex araneus* *Sorex coronatus* *Crocidura olivieri* *Crocidura grandiceps* *Crocidura attenuata* *Crocidura lasiura*	Germany Germany Côte d'Ivoire Sierra Leone China China	PCR + sequencing	liver	Rasche et al., 2019 Nie et al., 2019
ssDNA[e]	*Piccovirales*	*Parvoviridae*	bocaparvovirus	*Suncus murinus*	China	PCR	faecal samples	Xiong et al., 2019
			rat adeno-associated virus	*Suncus murinus*	China	only PCR, no sequences	not specified	Xiong et al., 2018b
	Lefavirales	*Anelloviridae*	todent torque teno virus	*Suncus murinus*	China	only PCR, no sequences	throat swab	Xiong et al., 2018a

[a] single-stranded ribonucleic acid of negative polarity, [b] single-stranded ribonucleic acid of positive polarity, [c] double-stranded ribonucleic acid, [d] double-stranded deoxyribonucleic acid, [e] single-stranded deoxyribonucleic acid

TEM: transmission electron microscopy; (RT-q) PCR: (reverse-transcription real-time) polymerase chain reaction; SGS: second-generation sequencing, either on an IonTorrent (Thermo Fisher) or Illumina platform (Illumina inc.); TGS: third-generation sequencing with Oxford Nanopore technology (ONT)

2.4.2 Pathogens in selected shrew species – Bacteria and endoparasites

Small mammals play also an important role as reservoirs and intermediate hosts for the transmission of non-viral pathogens such as bacteria and others (**Figure 3** and **Table 3**)**.** The four species of white-toothed shrews examined in this thesis have been identified as carriers of arthropod-borne pathogens. *Borrelia burgdorferi* was found in Romania (Kalmár et al., 2019), *Anaplasma phagocytophilum* in Spain and Romania (Barandika et al., 2007; Matei et al., 2018), and *Bartonella refiksaydamii* in Turkey (Celebi et al., 2021). Additionally, *Toxoplasma gondii* was reported from Switzerland (Pardo Gil et al., 2023) (**Table 3**). However, profound reports from investigations in Germany are scarce.

Table 3 Current knowledge on bacteria, protozoa, nematodes and trematodes detected in white-toothed shrews. Publicly accessible literature was searched via 'PubMed' until July 2023 utilizing the keywords '*Crocidura'*, 'white-toothed shrews', or '*Suncus'* in combination with 'pathogen', 'metagenome', 'bacteria' or 'endoparasite'. The classification is according to the currently internationally accepted taxonomy, whilst descriptions of pathogen species followed those proposed in the respective publication.

Type	Phylum: Family	*Species*	Detected in	Country of description	Method	Organ	References
Bacteria	Spirochaetota: Leptospiraceae	*L. kirschneri*	*Crocidura russula*	Germany	*secY* SLST	kidney	Mayer-Scholl et al., 2014
		L. kirschneri	*Crocidura leucodon*	Germany	*lipl*32 PCR	kidney	Jeske et al., 2021
		Leptospira spp.	*Crocidura russula*	Israel	staining	kidney	Torten et al., 1972
		L. kirschneri	*Crocidura goliath*	Gabon	16sRNA qPCR + *lipl*32 PCR	not specified	Mangombi et al., 2021
		L. borgpetersenii Leptospira spp.	*Suncus murinus*	Sri Lanka	*lipl*32 qPCR + MLST	kidney	Sluydts et al., 2022
		L. tipperaryensis	*Crocidura russula*	Ireland	*secY* PCR + SGS	kidney	Nally et al., 2016; Vincent et al., 2019
	Spirochaetota: Borreliaceae	*Borrelia burgdorferi* (s.l.)	*Crocidura leucodon* *Crocidura suaveolens*	Romania	*5S-23S* rDNA IGS + *ospA* PCR	heart, liver	Kalmár et al., 2019
		Borrelia spp.	*Suncus murinus* *Crocidura watasei*	Japan	cultured strains + *5S-23S* rDNA IGS PCR	ear	Masuzawa et al., 2004
		B. japonica	*Crocidura dsinezumi* *Sorex unguiculatus* *Sorex caecutiens*	Japan	culture + rRNA RFLP PCR	ear	Nakao et al., 1994
	Pseudomonadota: Yersiniaceae	*Yersinia pestis*	*Suncus murinus*	Madagascar	SGS	spleen	Rahelinirina et al., 2017
	Pseudomonadota: Ehrlichiaceae	*Anaplasma phagocytophilum*	*Crocidura russula* *Crocidura suaveolens*	Spain Romania	*msp*2 PCR *rrs* PCR	tissue pools; spleen	Barandika et al., 2007; Matei et al., 2018
		A. platys	*Crocidura lasiura*	Republic of Korea	16S rRNA PCR + specific Primer	spleen	Chae et al., 2008
	Pseudomonadota: Ehrlichiaceae	*Ehrlichia chaffeensis*	*Crocidura lasiura*	Republic of Korea	16S rRNA PCR+ specific primer	spleen	Chae et al., 2008
	Pseudomonadota: Bartonellaceae	*Bartonella refiksaydamii*	*Crocidura suaveolens*	Turkey	HTS + MLST	blood	Celebi et al., 2021
		B. florenciae	*Crocidura russula*	France	culture + MLST + HTS	spleen	Mediannikov et al., 2013

Table to be continued.

Type	Phylum: Family	*Species*	Detected in	Country of description	Method	Organ	References
Bacteria		*B. tribocorum*	*Suncus murinus*	Cambodia Taiwan Singapore	qPCR + SGS culture; *gltA* PCR *rpoB, nuoG, 16S rRNA PCR*	blood spleen	Hadjadj et al., 2018; Hsieh et al., 2010; Neves et al., 2018
		B. ratttimassiliensis	*Suncus murinus*	Taiwan	culture + *gltA* PCR	blood	Hsieh et al., 2010
		Bartonella spp.	*Crocidura lasiura*	Korea	16S rRNA + 23S rRNA + *gro*EL PCR	spleen	Kim et al., 2005
		Candidatus Bartonella crocidura	*Crocidura lasiura*	China	*gltA + rpoB* PCR	spleen	Zhang et al., 2022a
		B. coopersplainsensis	*Suncus murinus*	Japan, Taiwan	culture; *gltA + rpoB* PCR	blood	Kim et al., 2016
		Bartonella spp.	*Crocidura shantungensis*	Taiwan	culture; *gltA + rpoB* PCR	blood	Kim et al., 2016
		Bartonella spp.	*Crocidura attenuata*	Taiwan	culture; *gltA + rpoB* PCR	blood	Lin et al., 2012
		B. henselae	*Suncus murinus*	Myanmar Nepal	*nuoG, gltA, 16S-23S rRNA ITS PCR* *rpoB* + *gltA* PCR	spleen kidney, liver	Böge et al., 2021 Gundi et al., 2010
		B. queenslandensis	*Suncus murinus*	Nepal	*rpoB* + *gltA* PCR	kidney, liver	Gundi et al., 2010
		B. rochalimae	*Suncus murinus*	Nepal	*rpoB* + *gltA* PCR	kidney, liver	Gundi et al., 2010
	Pseudomonadota: Alphaproteobacteria (class) Rickettsiaceae	*Rickettsia japonica, R. rickettsia, R. typhi*	*Suncus murinus*	Taiwan	*ompB* + *gltA* PCR	liver, spleen, kidney	Kuo et al., 2015
	Bacillota: Clostridiaceae	*Clostridium perfringens*	*Suncus murinus*	India	isolation	faecal swabs	Milton et al., 2022
	Actinomycetota: Mycobacteriaceae	*Mycobacterium* spp.	*Crocidura hirta*	Tanzania	PCR for *M. tuberculosis* complex + culture	organ pool: liver, spleen, lung, mesenteric lymph node	Durnez et al., 2008
	Thermodesulfobacteriota: Desulfovibrionaceae	*Lawsonia intracellularis*	*Crocidura suaveolens*	Czech Republic	LIA + LIB PCR, LIC + LID PCR	intestinal mucous membrane	Friedman et al., 2008

Table to be continued.

Type	Phylum: Family	*Species*	Detected in	Country of description	Method	Organ	References
Protozoa	Euglenozoa: Trypanosomatida	*Leishmania infantum*	*Crocidura russula*	Spain	18S rRNA qPCR	spleen	Millán 2018
	Apicomplexa: Sarcocystidae	*Toxplasma gondii*	*Crocidura olivieri* *Crocidura russula* *Crocidura attenuata*	Benin Switzerland China	qPCR + MLST	heart, brain, muscle, liver	Etougbétché et al., 2022; Pardo Gil et al., 2023; Wang et al., 2019
	Apicomplexa: Sarcocystidae	*Sarcocystis attenuati*	*Crocidura attenuata*	China	LM + TEM + 18S rDNA PCR + MLST	muscle tissue	Hu et al., 2022
	Apicomplexa: Babesiidae	*Babesia microti*	*Crocidura horsfieldii*	Taiwan	SSUrDNA PCR	blood	Saito-Ito et al., 2008
	Apicomplexa: Hepatozoidae	*Hepatozoon spp.*	*Crocidura suaveolens*	Afghanistan	18S rRNA PCR + *Hepatozoon*-specific Adel1 PCR	spleen, liver, kidney	Schotte et al., 2023
	Euglenozoa: Trypanosomatidae	*Trypanosoma sapaensis*	*Crocidura dracula*	Vietnam	SSU rDNA + gGAPDH PCR	blood	Mafie et al., 2019
		T. crocidurae	*Crocidura suaveolens* *Crocidura russula*	Czech Republic Portugal Germany	not specified LM + isolation	not specified blood	Sebek 1975 Santos-Gomes et al., 1993 Krampitz 1959
Nematodes	Nematoda: Capillariidae	*Capillaria splenaecum*	*Crocidura russula*	Spain	LM: H.E. Staining	organs with macroscopic lesions	Millán et al., 2014
		C. crociduri	*Crocidura leucodon*	Iran	LM: Giemsa staining	blood	Yousefi et al., 2017
		C. hokkaidensis	*Crocidura leucodon*	Iran	LM: Giemsa staining	blood	Yousefi et al., 2017
Trematodes	Platyhelminthes: Schistosomatidae	*Schistosoma mansoni*	*Crocidura olivieri*	Kenya	LM + *cox*1 DNA PCR	hepatic port + liver	Hanelt et al., 2010
		Schistosoma kisumuensis	*Crocidura* spp.	Kenya	LM + *cox*1 DNA PCR	hepatic port + liver	Hanelt et al., 2010
	Platyhelminthes: Echinochasmidae	*Echinochasmus japonicas*	*Crocidura lasiura*	Republic of Korea	LM	gastrointestinal content	Chai et al., 2009
	Platyhelminthes: Taeniidae	*Echinococcus multilocularis*	*Crocidura gmelini*	Iran	*nad*1 PCR	liver	Beiromvand et al., 2013
	Platyhelminthes: Hymenolepidiae	*Staphylocystis biliarius*	*Crocidura russula*	Spain	LM	intestinal content	Mas-Coma and Jourdane 1977

LM: light microscopy; TEM: transmission electron microscopy; (q)PCR: (real-time) polymerase chain reaction; HTS: high-throughput sequencing; SGS: second-generation sequencing; MLST: multi-locus sequence typing; SLST: single-locus sequence typing; H.E. staining: hematoxylin-eosin staining

2.4.3 Genotyping of bacteria

Classification of bacteria was traditionally based on phenotypic characteristics such as Gram staining, growth requirements, and biochemical tests. The species-level phylogenetic relationship can be determined by 16S rRNA analysis, which is a highly conserved genome region. However, this stability also limits its discriminatory power to distinguish related species (Fouts et al., 2016). Consequently, additional genotyping schemes have been developed to identify genomo and sequence types (ST) to improve discrimination. This is based on sequence differences within one or multiple housekeeping genes (locus), which allow genome wide comparison. Molecular typing is a useful tool to differentiate between strains within a single species or subspecies (Perez et al., 2011). This allows the investigation of the epidemiological context of infectious diseases on a molecular level, both locally and globally (Ahrens and Pigeot 2014). This concept will be presented exemplarily for *Leptospira* spp.

Leptospira spp. are worldwide occurring bacteria with currently 64 named species, which have broad host spectra including humans, domestic animals and wildlife (Vincent et al., 2019). Some species have thus far only been isolated from the environment, and their clinical potential has not yet been characterized. *Leptospira* exposure occurs by direct contact to a positive animal or indirect through contact to urine-contaminated water and soil (Ko et al., 2009) (**Figure 3**). Humans, as dead-end hosts, may experience a wide range of clinical manifestation of leptospirosis. Symptoms range from mild-flu like to severe organ failure (Morbus Weil), and encephalitis (Bharti et al., 2003), which, if left untreated, may be lethal (< 10% lethality rate) (Yanagihara et al., 2007; Meerburg et al., 2009; Costa et al., 2015). As the life cycle relies on water, good sanitary standards have the potential to prevent infection, but it remains a major human and animal health concern particularly in resource poor (tropical) countries (Reis et al., 2008; WHO-SEARO 2009; Costa et al., 2015). In Germany, the risk of contracting a *Leptospira* infection is elevated during occupational activities involving agriculture and forestry (Bharti et al., 2003; Desai et al., 2009), recreational pursuits such as water sports and gardening (Jansen et al., 2005; Mayer-Scholl et al., 2014), and after periods of heavy flooding and rainfall (Lau et al., 2010). Nonetheless, outbreaks are typically sporadic. In Germany, dog vaccines are attainable against various strains of *Leptospira* as recommended by the ‘Ständige Impfkomission” of the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Germany (StIKo Vet 2016). Eight pathogenic *Leptospira* spp. are known (Vincent et al., 2019). The described *Leptospira* species are subdivided into three groups: pathogenic, intermediate and non-pathogenic or saprophytic group (Fouts et al., 2016). However, Vincent et al., (2019) proposed a new nomenclature distinct from the assessment of virulence characteristics, which still remain to be determined for many species, with four phylogenetically distinct subclades (P1, P2, S1, S2).

Single-locus sequence typing (SLST) for example based on the *secY* gene, coding for a transmembrane protein, permits the differentiation of species, but accurate identification may be challenged by the observed high genetic diversity between pathogenic *Leptospira* species. In addition, multi-locus sequence typing (MLST) targeting nucleotide differences across multiple genes of the bacterial genome has advanced to determine sequence types. The scheme implemented by Boonsilp et al., (2013) targets seven genes. It is combined with a public database and website to provide up to date information. MLST may be utilized in the identification of maintenance hosts, during outbreaks and for further epidemiological investigations. Rodents and small mammals have been identified as main reservoirs of *Leptospira* and studies by e.g. Fischer et al., (2018) revealed a complex sequence type pattern across multiple small mammal species in Germany. Different *Leptospira* spp. were identified in white-toothed shrews from Germany, Ireland, and worldwide (**Table 3**).

2.4.4 Notifiable zoonotic and enzootic diseases in Germany

Reporting human leptospirosis to the Robert Koch Institute is mandatory in Germany. In 2021, Germany's notification rate was 0.2 per 100,000 population, which corresponds to the mean rate within the European Union (ECDC 2023).

Notifiable zoonotic and enzootic diseases are monitored and regulated by the German protection against infection act ('Infektionsschutzgesetz', n=66 (IfSG, revised 7/17/2023)) for humans. For animals, the German regulation on notifiable and reportable diseases ('Tierseuchenanzeigeverordnung', n=53 (TierSeuchAnzV, revised 3/31/2020) and 'Verordnung über meldepflichtige Tierkrankheiten', n=26 (TKrMeldpflV, revised 2/11/2011), respectively) are in place. Important viral and bacterial pathogens with an established small mammal reservoir mentioned are: Monkeypox virus (MPV), tick-borne encephalitis virus (TBEV), hantaviruses (Puumala virus (PUUV), Seoul virus (SEOV)), zoonotic bornaviruses (variegated squirrel bornavirus 1 (VSBV-1), BoDV-1)), HEV, Lassa virus (LASV), Marburg virus (MARV), *Leptospira* spp., *Coxiella burnetii*, *Francisella tularensis*, *Yersinia pestis*. Numbers of reported human cases of selected pathogens with a known or suspected small mammal reservoir are demonstrated in **Figure 5**.

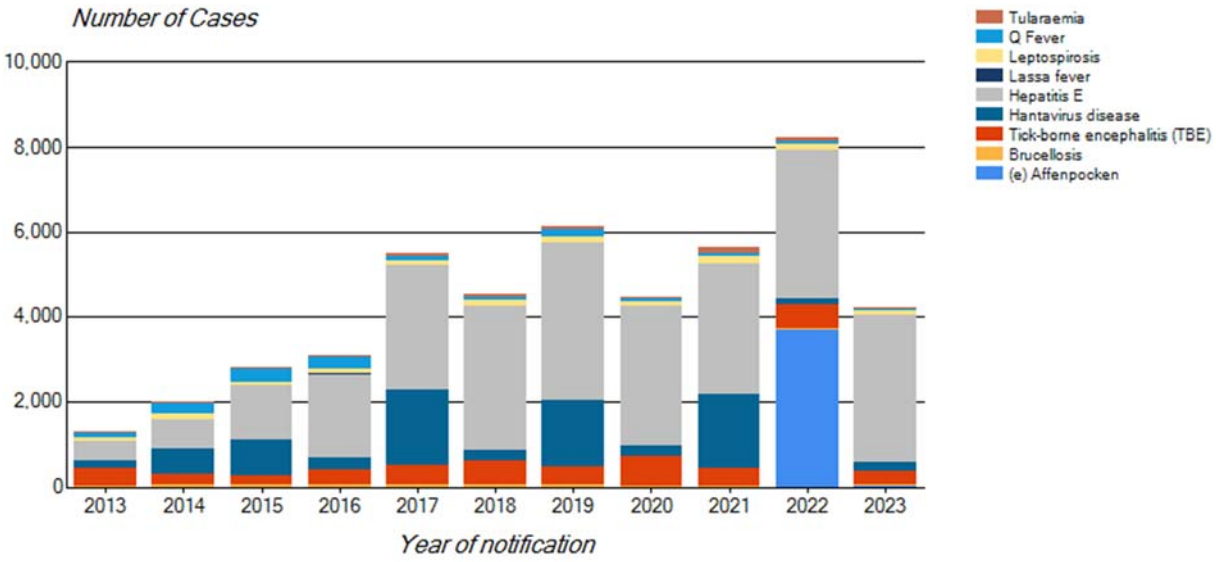

Figure 5 Human infections of selected pathogens with a known or suspected small mammal reservoir in Germany reported to the Robert Koch Institute between 2013 and 2023. »Robert Koch Institute: SurvStat@RKI 2.0, https://survstat.rki.de, deadline: 13/09/2023« (RKI 2023)

Detailed information on important viral zoonoses in the context of One Health in Germany, for which small mammals play an important role in the transmission cycle (PUUV, Dobrava virus (DOBV), SEOV, BoDV-1, VSBV-1, TBEV, HEV), can be found in the following review article. Figure and table numbering are according to the published chapter and references are presented in the journal style and do not appear in the reference section

Review 1: Virale Zoonosen in Deutschland aus der One Health-Perspektive

Virale Zoonosen in Deutschland aus der One Health-Perspektive

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Virale Zoonosen in Deutschland aus der One Health-Perspektive

Vorbemerkung

One Health ist ein „kollektiver, vereinender Ansatz, der darauf abzielt, die Gesundheit von Menschen, Tieren und Ökosystemen nachhaltig ins Gleichgewicht zu bringen und zu optimieren. Er erkennt an, dass die Gesundheit von Menschen, Haus- und Wildtieren, Pflanzen und der weiteren Umwelt (einschließlich der Ökosysteme) eng miteinander verbunden und voneinander abhängig sind. Der Ansatz mobilisiert verschiedene Sektoren, Disziplinen und Gemeinschaften auf unterschiedlichen Ebenen der Gesellschaft“ [1]. Sektorübergreifend bedeutet in diesem Zusammenhang, dass die Zusammenarbeit und der Austausch zwischen verschiedenen Sektoren, wie z. B. Gesundheitswesen, Veterinärmedizin, Umweltschutz, Landwirtschaft und Wissenschaft, notwendig sind, um eine umfassende Sicht auf die Gesundheit zu gewährleisten und effektive Lösungen für Gesundheitsprobleme zu entwickeln. Darüber hinaus beinhaltet es eine Zusammenarbeit verschiedener Regierungsbehörden, Nichtregierungsorganisationen (NGOs), internationaler Organisationen und der Privatwirtschaft, um gemeinsame Ziele im Bereich der Gesundheit zu erreichen. Durch diese sektorübergreifende Zusammenarbeit können Synergien geschaffen werden, um Gesundheitsprobleme zu lösen und eine nachhaltige Gesundheitsentwicklung zu fördern.

Bei der Erforschung, Prävention sowie Bekämpfung von viralen Zoonosen ist es nicht zielführend, einen isolierten Blick auf die Erkrankung beim Menschen oder bei Tieren, einschließlich der tierischen Erreger-Reservoir-Systeme zu werfen. Vielmehr sollte hierbei – wie im vorliegenden Beitrag beschrieben – der holistische „One Health-Ansatz“ zur Anwendung kommen. Der Begriff „Tiergesundheit“ umfasst Nutz- und Wildtiere. „Umweltgesundheit“ umfasst die abiotische und biotische Umwelt, inkl. der Kleinsäugerreservoirwirte, wie Nagetiere und Spitzmäuse, und Vektoren, wie Zecken und Stechmücken, die an der Erregerübertragung beteiligt sind (Abb. 1).

Charakteristisch für virale Zoonosen ist die dauerhafte Verbreitung des Erregers in bestimmten Tierspezies, den sogenannten Reservoirwirten. In Reservoirwirten führt die Infektion aber nicht immer zu einer Erkrankung. Reservoirwirte tolerieren die Infektion häufig, ohne eine klinische Symptomatik zu entwickeln, da der Beziehung Erreger-Reservoirwirt vielfach eine lange Koevolutionsgeschichte vorausgegangen ist. Springt der Erreger nun auf eine andere Spezies als den Reservoirwirt über (z. B. den Menschen), so wird das als Spillover bezeichnet, der neue Wirt auch als „Fehlwirt“ (Dead-end Host). Findet ein solches Spillover-Event statt, so kann dies ganz unterschiedliche Folgen für den Fehlwirt haben, die von Infektionsresistenz bis hin zu schweren oder letalen Krankheitsverläufen reichen können [2–5].

Einleitung

Die COVID-19-Pandemie und das erstmals auch in Europa gehäufte Auftreten von Mpox-Erkrankungen (ursprünglich: Affenpocken) haben die Verletzlichkeit der Bevölkerung für aus dem Tierreich stammende Krankheitserreger deutlich werden lassen [6–9]. Darüber hinaus haben in den vergangenen Jahren weitere virale Zoonoseerreger große Aufmerksamkeit erfahren: So wurde im Jahr 2022 in einer kleinen Gemeinde in Bayern erstmalig eine lokale Häufung von letalen Enzephalitiden bekannt, die auf Infektionen mit dem Borna Disease Virus 1 (BoDV-1) zurückgeführt werden konnten [10]. Auch die Zahl der erfassten Hepatitis E-Erkrankungsfälle ist in den vergangenen Jahren in Deutschland fast kontinuierlich angestiegen [11]. In regelmäßigen Abständen treten zudem

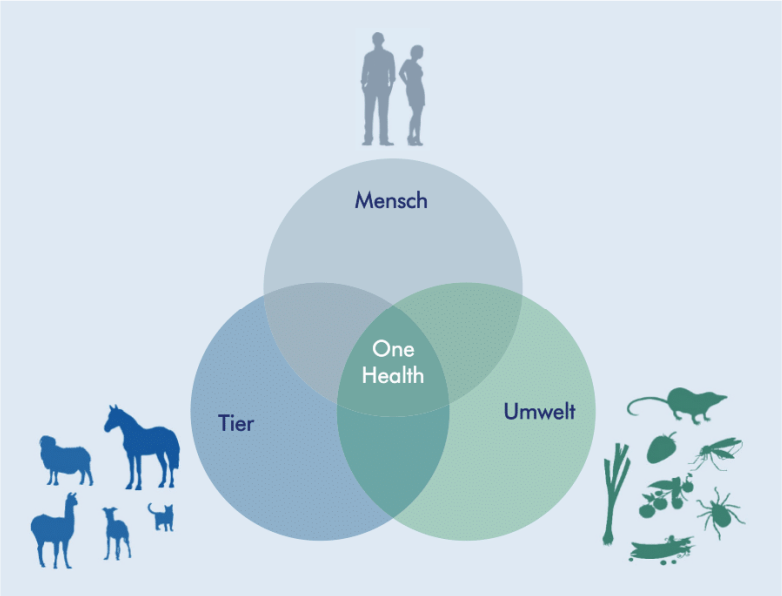

Abb. 1 ▲ Schematische Darstellung des One Health-Ansatzes. Quelle: eigene Abbildung

regionale Häufungen von Hantavirus-Erkrankungsfällen (bekannt als so genannte „Ausbruchsjahre") auf [12]; kürzlich wurde sogar von einer Hantavirus-Erkrankung durch Heimratten berichtet [13]. Seit 2018 kommt das von Mücken übertragene West-Nil-Virus (WNV) in bestimmten Regionen Deutschlands vor (vorrangig in den östlichen Gebieten) und hat sich dort fest etabliert [14]. Außerdem nehmen Erkrankungen durch das Frühsommer-Meningoenzephalitis-Virus (FSME-Virus) in Deutschland seit Jahren kontinuierlich zu. Das Verbreitungsgebiet des Erregers dehnt sich dabei nach Norden und auch im Osten Deutschlands aus. Auch lebensmittelübertragene FSME-Erkrankungen sind kürzlich in die Schlagzeilen geraten [15]. Hinzu kommen vereinzelte Spillover-Infektionen vom Reservoir auf andere Tierarten, die jedoch ohne oder nur mit begrenzter Zirkulation in der jeweiligen Tierart verbunden sind [16], wie der aktuelle Bericht über eine letale Infektion eines Affen in einem zoologischen Garten mit dem lymphozytären Choriomeningitis-Virus (LCMV) veranschaulicht [17].

Andere für den Menschen gefährliche zoonotische Viren, wie die Lyssaviren (Tollwutvirus und Fledermaus-Tollwutviren) führen derzeit glücklicherweise nur sehr selten zu humanen Infektionen in Deutschland, beziehungsweise werden nur als aus dem Ausland importierte Fälle berichtet [18]. Eine besondere Bedeutung haben die zoonotischen Influenzaviren des Vogels (aviäre Influenzaviren) und des Schweins (Schweineinfluenzaviren), insbesondere auch als Vorläufer von pandemischen Influenzaviren. Während in Deutschland glücklicherweise noch keine humanen Fälle mit aviären Influenzaviren beobachtet wurden, kommt es vereinzelt zur Infektion des Menschen mit Schweineinfluenzaviren.

Die mit den hier aufgelisteten Zoonoseerregern verbundenen Forschungsfragen sowie die Implementierung von Bekämpfungs- und Präventionsmaßnahmen lassen sich nur in einer sektorübergreifenden, transdisziplinären Betrachtungsweise im Sinne des One Health-Konzepts erfolgreich angehen. Ziel dieses Artikels ist daher die Darstellung der Herausforderungen und Wissenslücken anhand von ausgewählten Beispielen meldepflichtiger viraler Zoonosen. Diese exemplarische Darstellung beinhaltet Hantaviren, Hepeviren, das West-Nil-Virus, das FSME-Virus, zoonotische Influenzaviren und Bornaviren. All diese Viren und die dadurch induzierten Erkrankungen reflektieren unterschiedliche Übertragungswege, verschiedene Erreger-Diversitäten und Reservoire sowie unterschiedliche Erkrankungen bei Mensch und Tier (◘ **Tab. 1**).

Hantaviren – Erkrankungshäufungen und Nagetiermassenvermehrung

Die Entdeckung der Hantaviren geht auf Untersuchungen nach dem Koreakrieg in den 1950er-Jahren zurück [19, 20]. Im Rahmen dieser Untersuchungen in Südkorea wurde das Hantaanvirus (HTNV) in seinem Tierreservoir, der Brandmaus (*Apodemus agrarius*), entdeckt. Gegenwärtig sind insgesamt 53 Hantavirusarten beschrieben worden [21], darunter auch hochvirulente Hantavirus-Spezies wie das Sin-Nombre-Virus (SNV) mit dem Reservoirwirt Hirschmaus (*Peromyscus maniculatus*) in Nordamerika. Umfangreiche Untersuchungen zum Auftreten von SNV-Infektionen zeigten die Rolle klimatischer Besonderheiten auf. So führen das regelmäßig auftretende Wetterphänomen El Niño und die damit verbundenen starken Regenfälle zu einer Zunahme der Hirschmauspopulation [22], was letztlich zu einem nachfolgenden Anstieg der Hantavirus-Infektionen führt. Eine Übersicht zu den Charakteristika von Hantaviren und der verursachten Erkrankungen findet sich in ◘ **Tab. 1**.

In Deutschland werden die meisten Hantavirus-Erkrankungen durch das Puumala-Orthohantavirus (PUUV) hervorgerufen; Infektionen mit diesem Virus sind schon längere Zeit bekannt. Dieses Virus wird ausschließlich von der Rötelmaus (*Myodes glareolus*, syn. *Clethrionomys glareolus*) als Erregerreservoir auf den Menschen übertragen [23]. Im Rahmen einer intensiven Zusammenarbeit mit Partnern aus forstlichen Institutionen, dem Julius Kühn-Institut und der Universität Bern wurde die Ursache für das begrenzte Vorkommen des PUUV in bestimmten Teilen Deutschlands aufgeklärt. Mutmaßlich wurde das PUUV während der nacheiszeitlichen Wiederbesiedlung Deutschlands und Westeuropas aus einem westlichen

Refugium der Rötelmaus eingeschleppt [24]. Die heterogene Verteilung des PUUV in Deutschland betrifft dabei nicht nur das Fehlen des PUUV in den Bundesländern Mecklenburg-Vorpommern, Brandenburg, Berlin, Sachsen-Anhalt, Sachsen sowie im östlichen Teil von Thüringen, sondern beispielsweise auch in Teilen Bayerns und Baden-Württembergs. Ungünstige Umweltbedingungen beeinträchtigen möglicherweise den Erhalt des PUUV in lokalen Reservoirpopulationen und können auch zum Aussterben von Viruslinien führen [25]. In Endemiegebieten wird eine Evolution des PUUV in den lokalen Rötelmauspopulationen beobachtet; dadurch lassen sich bestimmte Viruslinien mit ihrer geografischen Verbreitung assoziieren [26–28]. Die Häufung von humanen Erkrankungen in bestimmten Jahren ist eng verknüpft mit einer starken Vermehrung der Rötelmaus. Diese Prozesse werden in Mittel- und Westeuropa durch die „Buchenmast", eine starke Fruchtbildung bei der Buche (*Fagus sylvatica*), getrieben [29]. In Deutschland wurden solche „Ausbruchsjahre" in den vergangenen Jahren im Zweijahresrhythmus beobachtet [11].

Die Übertragung des PUUV und vermutlich aller mit Säugetieren assoziierten Hantaviren erfolgt vor allem indirekt, z. B. durch das Einatmen kot- oder urinkontaminierten Staubs (**Abb. 2**). Unter Laborbedingungen bleibt die Tenazität des Hantavirus über mehrere Wochen erhalten [30]. Darüber hinaus geht man von einer Übertragung durch Bisse infizierter Nagetiere aus [31]. Weitere humane Infektionen sind in Deutschland auf die Hantavirusarten Dobrava-Belgrad-Virus (DOBV), Genotyp Kurkino, mit der Brandmaus (*Apodemus agrarius*) als Reservoir, das Tulavirus mit der Feldmaus (*Microtus arvalis*) als bevorzugtem Reservoir und ein Heimratten-assoziiertes Seoulvirus (SEOV) zurückgeführt worden [13, 32, 33]. Neben diesen Nagetier-assoziierten zoonotischen Hantaviren sind in den vergangenen Jahren weitere Hantaviren in anderen Nagetieren, aber auch in Spitzmäusen, Maulwürfen und Fledermäusen entdeckt worden (**Tab. 2**).

Zusammenfassung · Abstract

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Virale Zoonosen in Deutschland aus der One Health-Perspektive

Zusammenfassung

COVID-19-Pandemie und gehäuftes Auftreten von Mpox-Erkrankungen (Affenpocken) außerhalb Afrikas haben die Verletzlichkeit der Bevölkerung für aus dem Tierreich stammende Krankheitserreger deutlich werden lassen. Darüber hinaus haben in den vergangenen Jahren weitere virale Zoonoseerreger an Bedeutung gewonnen. Der vorliegende Übersichtsartikel beleuchtet anhand von 6 meldepflichtigen viralen Zoonoseerregern beispielhaft die Notwendigkeit der One Health-Herangehensweise, um die Epidemiologie der Erkrankungen verstehen zu können und Handlungsempfehlungen für den öffentlichen Gesundheitsdienst abzuleiten. Dabei wird die Bedeutung von Umweltfaktoren, Reservoiren und Vektoren betont, die Erkrankungen bei Nutz- und Wildtieren werden analysiert sowie das Auftreten und die Häufigkeit von Erkrankungen bei der Bevölkerung beschrieben. Die hier ausgewählten Erreger unterscheiden sich in den Reservoiren und der Rolle von Vektoren für die Übertragung, den Auswirkungen der Infektionen auf landwirtschaftliche Nutztiere und den beim Menschen beobachteten Krankheitsbildern. Neben bereits lange in Deutschland bekannten Zoonoseerregern werden auch Erreger betrachtet, die erst kürzlich eingetragen wurden bzw. deren Zoonosepotenzial vor Kurzem erstmals gezeigt worden ist.

Bei den hier behandelten Erregern gibt es nach wie vor deutliche Wissenslücken zu den Übertragungswegen. Zukünftige One Health-basierte Untersuchungen werden zu deren weiterer Aufklärung und somit zur Entwicklung von Präventionsmaßnahmen beitragen. Die ganzheitliche Herangehensweise beinhaltet nicht zwangsläufig eine Fokussierung auf virale Erreger/Erkrankungen, sondern beinhaltet auch die Frage der Wechselwirkungen von viralen, bakteriellen und anderen Erregern, inkl. der Antibiotikaresistenz und der Wirtsmikrobiome.

Schlüsselwörter

Hantaviren · Hepeviren · Bornaviren · Flaviviren · Influenzaviren

Viral zoonoses in Germany: a One Health perspective

Abstract

The COVID-19 pandemic and the increasing occurrence of monkeypox (mpox) diseases outside Africa have illustrated the vulnerability of populations to zoonotic pathogens. In addition, other viral zoonotic pathogens have gained importance in recent years.

This review article addresses six notifiable viral zoonotic pathogens as examples to highlight the need for the One Health approach in order to understand the epidemiology of the diseases and to derive recommendations for action by the public health service. The importance of environmental factors, reservoirs, and vectors is emphasized, the diseases in livestock and wildlife are analyzed, and the occurrence and frequency of diseases in the population are described. The pathogens selected here differ in their reservoirs and the role of vectors for transmission, the impact of infections on farm animals, and the disease patterns observed in humans. In addition to zoonotic pathogens that have been known in Germany for a long time or were introduced recently, pathogens whose zoonotic potential has only lately been shown are also considered.

For the pathogens discussed here, there are still large knowledge gaps regarding the transmission routes. Future One Health-based studies must contribute to the further elucidation of their transmission routes and the development of prevention measures. The holistic approach does not necessarily include a focus on viral pathogens/diseases, but also includes the question of the interaction of viral, bacterial, and other pathogens, including antibiotic resistance and host microbiomes.

Keywords

Hantaviruses · Hepeviruses · Bornaviruses · Flaviviruses · Influenza viruses

Tab. 1 Übersicht zu den Eigenschaften in Deutschland bekannter ausgewählter viraler Zoonoseerreger

Erreger (Anzahl Erregerarten in DE)	Erkrankung	Symptome	Humane Meldefälle in DE (*n*)	Letalität	Erkrankung bei Tieren	Übertragung	Umweltstabilität	Referenz
Hantaviren (mindestens 9; ◘ **Tab. 2**)	*In Europa und Asien:* Hämorrhagisches Fieber mit renalem Syndrom (HFRS) *In Amerika:* Hantavirus-induziertes kardiopulmonales Syndrom (HCPS)	Grippeähnliche Symptome, Fieber, Blutungen, Niereninsuffizienz, Proteinurie, Anurie, Schock	Fallzahl schwankt jährlich. Im Zeitraum 2018–2022 wurden durchschnittlich 780 Fälle übermittelt (Range: 141–1719)	HFRS: 0,1–12,0 % HCPS: bis zu 35 %	Symptomlos im Reservoir	*Indirekt:* durch Aufwirbeln virushaltiger Stäube von Reservoirexkrementen *Direkt:* durch Biss	Unbekannt, unter experimentellen Bedingungen 10 bis 15 Tage bei Raumtemperatur; bei 4 °C bis zu 18 Tage	[11, 30, 118]
Hepatitis E-Virus und verwandte Viren (2; ◘ **Tab. 3**)	Hepatitis E	Akut: grippeähnliche Symptome, Oberbauchschmerzen, Ikterus Chronisch: Leberzirrhose	Von 2011 bis 2018 stark ansteigende Fallzahlen (von 238 auf 3400 Fälle). Seit 2018 relativ stabile Fallzahlen mit durchschnittlich 3400 Fällen pro Jahr (Range: 3079–3729)	0,5–4 %	Symptomlos	Hauptsächlich durch den Verzehr ungenügend erhitzter Fleischprodukte von Haus- und Wildschwein. Auch über kontaminiertes Trinkwasser, Blutprodukte und direkten Tierkontakt möglich	Sehr stabil bei pH 2–9 und Salzkonzentrationen bis 20 % NaCl; stabil nach Trocknung (nur 1 log Verringerung nach 8 Wochen auf Plastik bei 4 °C, stärkere Inaktivierung auf Holz)	[11, 40, 119, 120]
Bornaviren (2; ◘ **Tab. 4**)	Progressive Enzephalitis	Fieber, Kopfschmerzen, Sprach- und Koordinationsstörungen, Koma	Erst seit März 2020 besteht eine Meldepflicht. Zwischen 2020–2022 sind dem RKI jährlich 5–7 akute Infektionen mit Borna Disease Virus 1 (BoDV-1) übermittelt worden	> 95 %	Borna'sche Krankheit bei Haustieren, insbes. Pferden, Schafen und Neuweltkameliden	BoDV-1 durch Spitzmäuse, Variegated Squirrel Bornavirus 1 (VSBV-1) durch gehaltene exotische Hörnchen; genauer Übertragungsweg unbekannt	Keine genauen Daten verfügbar, aber vermutlich gering	[63, 64, 121]
West-Nil-Virus (WNV; 1)	West-Nil-Fieber, West-Nil-Enzephalitis	Grippeähnliche Allgemeinsymptomatik, Kopfschmerz, Übelkeit, Erbrechen, Nackensteifigkeit, Bewusstseinsänderung, Koordinationsstörungen, Lähmungen; schwere Verlaufsformen im höheren Alter gehäuft	2018 sind autochthone Übertragungen von WNV erstmals in DE nachgewiesen worden. Im Zeitraum 2018–2022 wurden jährlich 5–21 WNV-Fälle an das RKI übermittelt, von denen ein Teil reiseassoziiert war	Bei Enzephalitis im höheren Alter bis 10 %	Meningitis, Enzephalitis bei Pferden	Stechmückenstiche; selten Kontakt mit virushaltigem Blut, Gewebe	Keine genauen Daten verfügbar, aber vermutlich gering	[18, 96, 100]

Tab. 1 (Fortsetzung)

Erreger (Anzahl Erregerarten in DE)	Erkrankung	Symptome	Humane Meldefälle in DE (*n*)	Letalität	Erkrankung bei Tieren	Übertragung	Umweltstabilität	Referenz
Frühsommer-Meningoenzephalitis-(FSME-)Virus (1); *5 Subtypen, davon der europäische, sibirische und fernöstliche Subtyp medizinisch am wichtigsten*	In Europa und Nordasien Meningitis, Enzephalitis, Enzephalomyelitis	Grippeähnliche Allgemeinsymptomatik, Kopfschmerz, Übelkeit, Erbrechen, Nackensteifigkeit, Bewusstseinsänderung, Koordinationsstörungen, Lähmungen	Jährlich schwankende Fallzahlen. Im Rekordjahr 2020 sind > 700 Fälle an das RKI übermittelt worden (Durchschnittl. FSME-Fallzahl 2018–2022: 540)	Europäischer Subtyp: 1–2 %; sibirischer Subtyp: ca. 5 %; fernöstlicher Subtyp: bis ca. 20 %	Meningitis, Enzephalitis bei Pferden, Affen, selten bei Hunden, vereinzelt bei Schafen	Zeckenstiche, selten Konsum roher Milch/Milchprodukte von infizierten Ziegen, Schafen, Kühen	Keine genauen Daten verfügbar; bei 4 °C in proteinhaltigen Lösungen (z. B. Milch) bis zu 2–3 Wochen	[11, 15, 78]
Lymphozytäres Choriomeningitis-Virus (1)	Verschiedene Krankheitsbilder darunter Meningitis, Enzephalitis	Grippeähnliche Allgemeinsymptomatik, Kopfschmerz, Übelkeit, Erbrechen, Nackensteifigkeit, Bewusstseinsänderung, Koordinationsstörungen, Lähmungen	Keine systematischen Angaben zu humanen Erkrankungsfällen verfügbar	Unbekannt	Häufig letale Hepatitis bei Neuweltprimaten	Übertragung über infektiöse Ausscheidungen von Hausmäusen und Hamstern	Unbekannt	[17]
Rabiesvirus (1)	Tollwut	U. a. Kopf-, Muskelschmerzen, Schluckstörungen, vermehrte Speichelbildung, erhöhte Reiz- und Erregbarkeit, Angstzustände, Lähmungen	Seit 2005 gab es 5 Tollwuterkrankungen in DE, hiervon 2 reiseassoziierte Infektionen und 3 Infektionen nach Erhalt eines Organs einer infizierten Spenderin	~ 100 %	Tollwut bei Säugetieren	Übertragung i. d. R. durch Bissverletzungen	gering	[18]
Zoonotische Influenzaviren (unbekannt)	Influenza-like Illness; Pneumonie	Grippeähnliche Allgemeinsymptomatik vergleichbar mit saisonaler Influenza. Bei aviärer Influenza häufig Beteiligung der unteren Atemwege	Zoonotische Influenza kommt in DE extrem selten vor. 2020 wurde ein Fall einer porcinen Influenza berichtet	Bei aviärer Influenza: 20–60 % je nach Subtyp	Geflügelpest: Je nach Virusstamm häufig letal verlaufende Erkrankung bei Vögeln und Geflügel; Schweineinfluenza: Akute Atemwegserkrankung bei Schweinen mit geringer Letalität	Übertragung durch Kontakt zu infizierten Vögeln oder Schweinen	gering	[18]

DE Deutschland, *RKI* Robert Koch-Institut

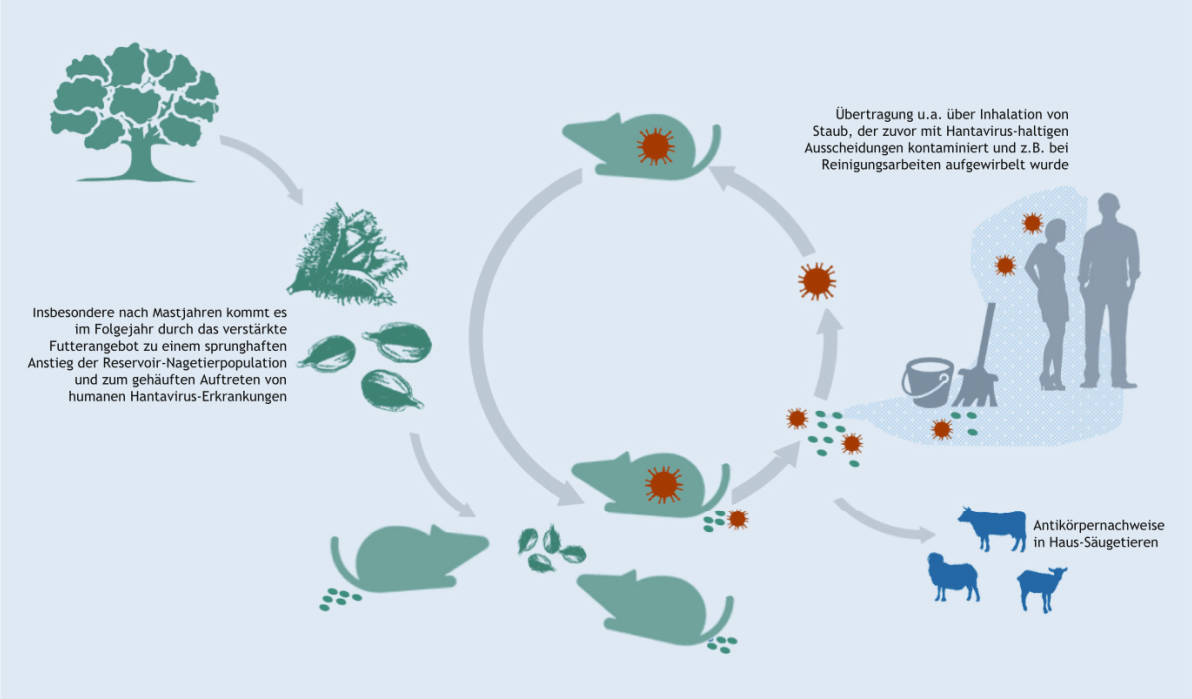

Abb. 2 ▲ Zoonotische Hantaviren wurden bisher ausschließlich bei Nagetieren gefunden. Die persistent infizierten Nagetiere scheiden den Erreger mit Urin, Kot und Speichel aus – die Übertragung in den Nagetierpopulationen und auf den Menschen erfolgt indirekt durch virushaltige Stäube oder direkt durch Biss. Der Mensch stellt einen Fehlwirt für das Virus dar. Die Häufigkeit humaner Infektionen ist von verschiedenen biotischen und abiotischen Umweltfaktoren abhängig. So kann die Buchenmast, d. h. eine sehr starke Fruchtbildung der Buche (*Fagus sylvatica*), im Folgejahr zu einer starken Vermehrung der Rötelmaus, dem Reservoirwirt des Puumalavirus, führen. (Quelle: eigene Abbildung)

Obgleich bei serologischen Studien Hantavirus-reaktive Antikörper in verschiedenen Haus- und Nutztieren nachgewiesen worden sind, gibt es bisher keine Hinweise auf Erkrankungen bei diesen Tieren [34].

Hepatitis E-Virus – Ein lebensmittelübertragener Zoonoseerreger

Das humanpathogene Hepatitis E-Virus (HEV, Spezies *Paslahepevirus balayani*; Familie *Hepeviridae*; [35]), wurde erstmals 1983 durch einen Selbstversuch nachgewiesen, bei dem sich der Arzt Mikhail Balayan eine filtrierte Stuhlprobe eines an einer neuartigen Hepatitis Erkrankten verabreichte [36]. Ab 1997 wurden auch in Schweinen HEV-Stämme gefunden, die denen des Menschen sehr ähnlich waren [37]. Experimentelle Infektionen von Haus- und Wildschweinen sowie molekularepidemiologische Nachweise von Virusübertragungen durch Fleisch- und Wurstwaren auf den Menschen wiesen später den zoonotischen Charakter der HEV-Infektion nach ([38]; ◘ **Abb. 3**). Eine zoonotische Übertragung von HEV ist vor allem auf die Genotypen HEV-3 und HEV-4 zurückzuführen. Daneben existieren die humanpathogenen Genotypen HEV-1 und HEV-2, welche nur von Mensch zu Mensch, hauptsächlich über fäkal kontaminiertes Trinkwasser, übertragen werden [38]. Weiterhin kann der zoonotische Genotyp HEV-7 von Dromedaren auf den Menschen übertragen werden [39]. In Deutschland spielt HEV-3 mit Abstand die größte Rolle; eine Übersicht zu weiteren hierzulande in Säugetieren vorkommenden HEV-ähnlichen Viren ist in ◘ **Tab. 3** zu sehen.

Die Infektion mit HEV kann beim Menschen eine akute Hepatitis hervorrufen. Infektionen mit HEV-1 bei Schwangeren sowie Infektionen mit allen humanpathogenen Genotypen bei Personen mit Lebervorschädigung können zu schweren und tödlichen Erkrankungen führen. Darüber hinaus stellen chronische Infektionen bei Transplantationspatienten, die zu einer lebensbedrohlichen Leberzirrhose führen können, ein zunehmendes Problem dar [40]. Ansonsten verlaufen die meisten HEV-Infektionen aber offensichtlich mild oder ohne klinische Symptome. Die Zahl der dem Robert Koch-Institut (RKI) gemeldeten Hepatitis E-Fälle zeigte in den vergangenen 10 Jahren einen deutlichen Anstieg, der aber vermutlich auf verbesserte und intensivierte Diagnostik zurückzuführen ist (◘ **Tab. 1**, [11]). Eine deutschlandweite Studie zeigte, dass bei durchschnittlich 15,3 % der Bevölkerung HEV-spezifische Antikörper nachweisbar sind [41].

Vor allem in Haus- und Wildschweinen in Deutschland, aber auch in geringerem Maße in Wildwiederkäuern, Kaninchen und anderen Tierarten, wurde

Tab. 2 In Deutschland nachgewiesene Hantaviren

Hantavirus	Reservoir	Zoonotisch	Verbreitung in Deutschland	Referenz
Puumalavirus	Rötelmaus (*Myodes glareolus*, syn. *Clethrionomys glareolus*)	Ja	Westliches, südliches und nordwestliches Deutschland	[24, 26, 27]
Dobrava-Belgrad-Virus	Brandmaus (*Apodemus agrarius*)	Ja	Östlicher Teil Deutschlands	[32, 122, 123]
Tulavirus	Feldmaus[a] (*Microtus arvalis*)	Ja	Gesamtes Deutschland	[33, 124]
Seoulvirus	Wanderratte (*Rattus norvegicus*)	Ja	Heimratten, nicht in Wildratten	[13, 125]
Tatenalevirus, Stamm Traemmerseevirus	Erdmaus (*Microtus agrestis*)	Unbekannt	Ein Nachweis in Brandenburg	[126]
Seewisvirus	Waldspitzmaus (*Sorex araneus*)	Unbekannt	Deutschlandweit	[127, 128]
Asikkalavirus	Zwergspitzmaus (*Sorex minutus*)	Unbekannt	An einem Fangort	[129]
Brugesvirus	Europäischer Maulwurf (*Talpa europaea*)	Unbekannt	An einem Fangort	[130]
Brnovirus	Großer Abendsegler (*Nyctalus noctula*)	Unbekannt	An 3 Orten	[131]

[a]Das Tulavirus wurde auch in verwandten Wühlmausarten molekular nachgewiesen, z. B. in der Erdmaus (***Microtus agrestis***); möglicherweise stellt die Erdmaus auch einen Reservoirwirt dar

HEV häufig nachgewiesen. Hierbei wurden Antikörperprävalenzen von 46,9 % in Hausschweinen [42] und 33,0 % in Wildschweinen [43] ermittelt. Die Tiere erkranken bei einer HEV-Infektion nicht, weshalb HEV-infizierte Tiere nicht bei der Schlachtung auffallen. Verschiedene Studien zeigen deshalb auch, dass in Lebensmitteln, die Schweinefleisch oder -leber enthalten, häufig HEV-RNA nachweisbar ist – so beispielsweise in 4,9 % der untersuchten Schweineleber-Proben [44], 15,0 % der Leberpaté-Proben [44] oder 20,0 % der Rohwurst-Proben [45] aus Deutschland. Stabilitätsuntersuchungen zeigen, dass eine Erhitzung der Lebensmittel das enthaltene HEV inaktivieren kann, jedoch nicht andere Arten der Haltbarmachung wie Pökeln, pH-Wert-Absenkung oder Trocknen [46–48]. Als Hauptübertragungsweg für HEV in Deutschland und Europa wird demnach der Verzehr von Fleischprodukten aus Haus- und Wildschweinen, die nicht ausreichend gegart worden sind, angesehen (**Abb. 3**). Andere Übertragungswege, beispielsweise über Blutprodukte, direkten Kontakt zu Tieren oder Umweltkontaminationen spielen demgegenüber wahrscheinlich eine untergeordnete Rolle.

Neuere Untersuchungen legen nahe, dass auch Kaninchen sowie Wander- und Hausratten eine Bedeutung bei der Übertragung von HEV oder HEV-ähnlichen Viren auf den Menschen haben können. In Kaninchen kommt ein besonderer Subgenotyp (HEV-3ra) vor, der auch in Deutschland schon mehrfach nachgewiesen wurde [49]. HEV-3ra wurde weltweit in Einzelfällen auch in humanen Hepatitis-Patienten nachgewiesen. In Wanderratten (*Rattus norvegicus*) aus Deutschland wurde im Jahr 2010 erstmals ein dem HEV verwandtes Virus identifiziert [50], das danach auch in vielen anderen Ländern vorgefunden wurde [51]. Wegen der fehlenden Übertragbarkeit dieses Ratten-HEV auf nichthumane Primaten durch experimentelle Inokulation wurde dieses Virus ursprünglich als nichtzoonotisch angesehen [52]. Kürzlich wurden allerdings in Hongkong, Kanada und Spanien mehrere humane Hepatitis-Fälle bekannt, die offensichtlich durch das Ratten-HEV hervorgerufen wurden [53]. Die genauen Übertragungswege der Ratten- und Kaninchenviren auf den Menschen sind bisher unbekannt und Gegenstand weiterer Untersuchungen. Ebenso muss in Zukunft geklärt werden, inwiefern HEV-ähnliche Viren, die in Feldmäusen (Ordnung Rodentia) und Fledermäusen (Ordnung Chiroptera) gefunden wurden (**Tab. 3**), auch ein Risiko der Übertragung auf den Menschen besitzen.

Bornaviren – selten, aber meist tödlich

Die Borna'sche Krankheit (Borna Disease, BD) wird durch BoDV-1 ausgelöst und wurde bereits im 19. Jahrhundert als „hitzige Kopfkrankheit" bei Pferden beschrieben [54, 55]. Sie ist eine zumeist tödlich verlaufende neurologische Erkrankung, die mit einer nichteitrigen, durch Immunpathogenese vermittelten Enzephalitis einhergeht. Neben Pferden erkranken gehäuft auch Schafe und Neuweltkameliden (Alpakas, Lamas); grundsätzlich empfänglich für BoDV-1 ist jedoch ein breites Spektrum von Säugetieren [54, 56, 57]. Das zoonotische Potenzial von BoDV-1 blieb lange Zeit unklar, da Nachweise von Infektionen beim Menschen fehlten. Die seit den 1980er-Jahren diskutierten Zusammenhänge von BoDV-1 und psychiatrischen Erkrankungen beim Menschen konnten nicht bestätigt werden bzw. stellten sich als Laborartefakte heraus [58–60].

Erst im Jahr 2018 wurde zweifelsfrei gezeigt, dass BoDV-1 auch beim Men-

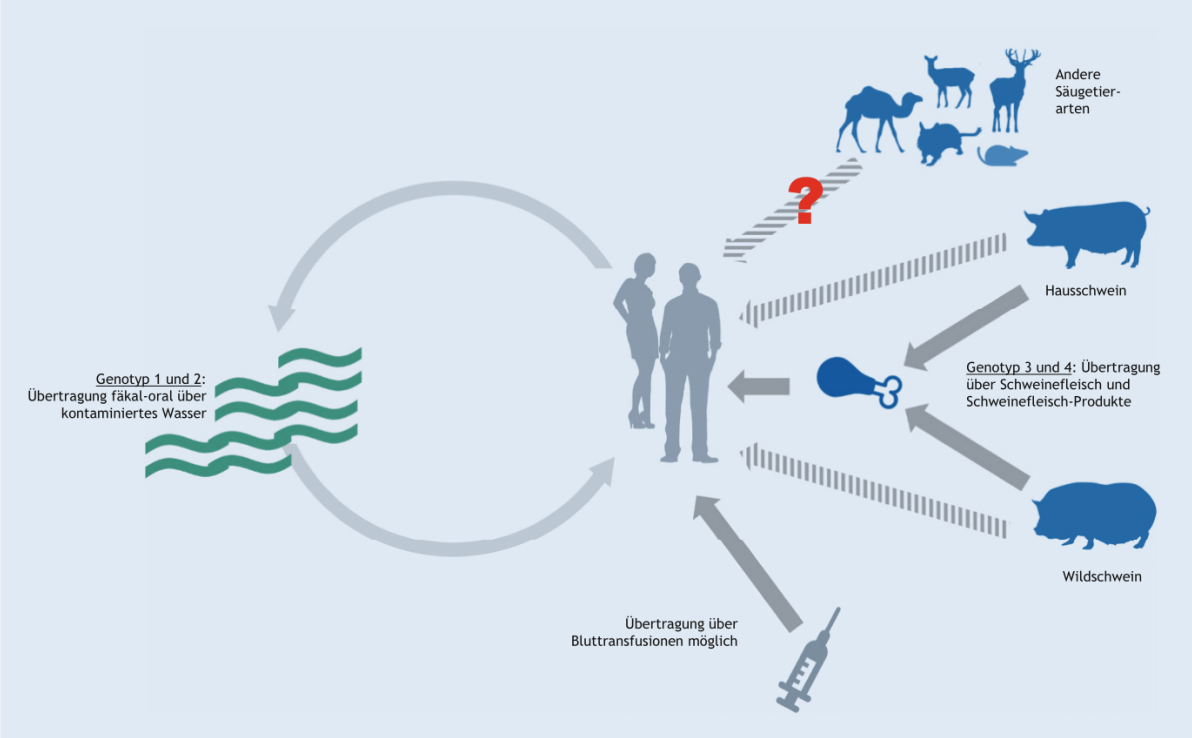

Abb. 3 ▲ Beim humanpathogenen Hepatitis E-Virus (HEV) werden nicht-zoonotische Genotypen (Genotypen 1 und 2, werden hauptsächlich über kontaminiertes Trinkwasser übertragen, linke Bildseite) von zoonotischen Genotypen (vor allem Genotypen 3 und 4, weitere Übertragungswege, rechte Bildseite) unterschieden. Reservoirwirte dieser zoonotischen Genotypen sind Wild- und Hausschwein (*Sus scrofa*), Kaninchen (*Oryctolagus cuniculus*) und weitere Säugetiere. Neben der alimentären Übertragung durch Genuss von ungenügend gegartem Fleisch und von Rohwurstprodukten wurden auch Übertragungen durch Bluttransfusionen berichtet. Die Häufung von Infektionen bei Tiermedizinern deutet auf weitere Übertragungswege hin. Bestätigt wurde diese Annahme beispielsweise durch den Nachweis der Wirkung des Tragens von Schutzhandschuhen beim Aufbrechen von Wildbret [138]. Neben den zoonotischen Genotypen 3 und 4 wurde in den vergangenen Jahren auch das Zoonosepotenzial des Genotyps 7 (aus Dromedaren) und des Ratten-Hepatitis E-Virus gezeigt. Quelle: eigene Abbildung

schen eine progressive Enzephalitis hervorrufen kann, die in mehr als 95 % der Fälle zum Tod führt [61, 62]. Symptomatisch äußert sich eine BoDV-1-Infektion beim Menschen initial meist durch Fieber und Kopfschmerzen, gefolgt von einer Reihe neurologischer Symptome (z. B. Sprach- und Koordinationsstörungen, Verwirrtheit, Wesensänderungen). Innerhalb weniger Tage bis Wochen fallen betroffene Personen in ein tiefes Koma und versterben in der Regel ([63]; ◘ **Tab. 1**).

Durch die Einführung einer Meldepflicht für Bornavirus-Infektionen bei Mensch und Tier im März 2020 ist zu erwarten, dass sich ein genaueres Bild zur Häufigkeit von humanen Erkrankungen abzeichnen wird. Zwischen 2020–2022 sind dem RKI jährlich 6–7 akute Fälle von BoDV-1-Enzephalitis übermittelt worden. Mit Stand Februar 2023 sind insgesamt 45 humane BoDV-1-Fälle erfasst worden, von denen ein Teil aus asserviertem Gewebematerial von Enzephalitisfällen unklarer Genese retrospektiv diagnostiziert wurde [63–65]. Auf Basis dieser Zahl ist von einer sehr niedrigen Inzidenz auszugehen. Momentan kann aber noch keine Aussage zum Manifestationsindex und zur entsprechenden Dunkelziffer getroffen werden: In Seroprävalenzstudien mit mehreren Hundert Teilnehmenden konnte lediglich eine einzelne seropositive Probe bei einer Tierärztin von der Schwäbischen Alb gefunden werden (u. a. [66, 67]).

Als Reservoirwirt von BoDV-1 wird gegenwärtig die Feldspitzmaus (*Crocidura leucodon*) angesehen, welche zu den Insektenfressern (Ordnung Eulipotyphla) gehört ([68, 69]; ◘ **Tab. 4**). Vermutlich tragen die Tiere das Virus lebenslang in sich, ohne daran zu erkranken, und scheiden es u. a. über Speichel, Urin, Kot und über die Haut aus [70]. Die genauen Übertragungswege innerhalb der Spitzmauspopulation sowie auf andere Wirte, wie Haussäugetiere oder den Menschen, sind bisher unbekannt (◘ **Abb. 4**). In Nichtreservoirwirten verhält sich das Virus strikt neurotrop. Es ist praktisch ausschließlich im zentralen Nervensystem (ZNS) sowie vereinzelt in Nervenzellen außerhalb des ZNS zu finden und wird daher von diesen Wirten auch nicht ausgeschieden. Sie fungieren somit als Fehlwirt für das Virus. Das bisher bekannte Endemiegebiet wird durch den BoDV-1-RNA-Nachweis primär in Re-

Tab. 3 In Deutschland bei Säugetieren nachgewiesene Hepatitis E-Viren (HEV) und HEV-ähnliche Viren

Hepevirus	Genotyp	Wirt	Zoonotisch	Geografische Verbreitung	Referenz
Humanes Hepatitis E-Virus *(Paslahepevirus balayani)*	Vor allem Genotyp 3	Wildschwein (*Sus scrofa*), Hausschwein, Kaninchen (*Oryctolagus cuniculus*) und weitere Säugetiere	Ja	Im gesamten Deutschland	[49, 119, 120]
Ratten-Hepatitis E-Virus (*Rocahepevirus ratti*)	N. d.[a]	Wanderratte (*Rattus norvegicus*)	Ja[b]	Im gesamten Deutschland	[50, 51, 132–134]
Feldmaus-Hepevirus	N. d.	Feldmaus (*Microtus arvalis*)	Unklar	An 4 Fangorten	[135]
Fledermaus-Hepeviren	N. d.	Breitflügelfledermaus (*Eptesicus serotinus*) Wasserfledermaus (*Myotis daubentonii*) Bechsteinfledermaus (*Myotis bechsteinii*)	Unklar	Einzelne positive Proben	[136]

[a]Mögliche Unterschiede zwischen den Genotypen des Ratten-Hepatitis E-Virus bezüglich des Zoonosepotenzials sind nicht bekannt

[b]Der Nachweis des Zoonosepotenzials des Ratten-Hepatitis E-Virus beruht auf dem molekularen Nachweis viraler RNA in Patienten in Hongkong, Kanada und Spanien; in Deutschland liegen bisher solche Daten nicht vor

N. d. nicht definiert

Tab. 4 In Deutschland gefundene humanpathogene Bornaviren

Art	Wirt	Zoonotisch	Erkrankung bei Tieren	Geografische Verbreitung in Deutschland	Referenz
Borna Disease Virus 1 (BoDV-1)	Feldspitzmaus *(Crocidura leucodon)*	Ja	Ja	Bayern, Thüringen, Sachsen, Sachsen-Anhalt, Brandenburg sowie z. T. angrenzende Bundesländer	[68, 69]
Bunthörnchen-Bornavirus 1 (Variegated Squirrel Bornavirus 1, VSBV-1)	Verschiedene exotische Hörnchenarten (Virus nur in Haltungen gefunden)	Ja	Bisher unbekannt	Nur bei Hörnchen-Haltungen in Deutschland; letzter Nachweis 2019	[71, 121, 137]

servoirwirten und Haussäugetieren definiert und erstreckt sich von Bayern, über Thüringen, Sachsen, Sachsen-Anhalt bis nach Brandenburg. Auch in der Ostschweiz, sowie Teilen Liechtensteins und Österreichs kommt BoDV-1 vor [58]. Umfangreiche Untersuchungen sind notwendig, um die exakte geografische Verbreitung des Virus festzustellen, wie eine Studie im Nordwesten Brandenburgs zeigte. Erstmalig wurde hier ein Vorkommen von BoDV-1 in dieser Region anhand von Erkrankungsfällen bei Alpakas sowie dem Nachweis von BoDV-1-RNA in einer Feldspitzmaus belegt [56].

Das Auftreten von 2 tödlichen Erkrankungsfällen in einer Gemeinde in Bayern 2019 und 2022 führte zu umfangreichen One Health-basierten Untersuchungen zu BoDV-1, die sowohl die Bevölkerung als auch potenzielle Kleinsäugerwirte, Bodenproben und Zecken beinhalteten [10]. Ein Fokus zukünftiger Untersuchungen sollte auf der weiteren Charakterisierung des Erreger-Reservoir-Systems, der Stabilität des Erregers in der Umwelt sowie der Ermittlung möglicher Übertragungswege liegen. So können zukünftig präzisere Empfehlungen zur Vermeidung dieser tödlichen Infektion gegeben werden.

Neben BoDV-1 gibt es mit dem Bunthörnchen-Bornavirus (Variegated Squirrel Bornavirus 1, VSBV-1) ein weiteres zoonotisches Bornavirus (**Tab. 1 und 4**). Dieses Virus wurde erstmals 2015 im Rahmen der Ermittlungen zu 3 tödlichen humanen Enzephalitisfällen beschrieben [71]. Die Entdeckung dieses Virus ist auf eine intensive Zusammenarbeit von Human- und Veterinärmedizin zurückzuführen [71], bei der eine Verbindung zwischen den humanen Fällen aufgrund der Gemeinsamkeit der Haltung von exotischen Hörnchen hergestellt werden konnte. Die darauffolgende Beprobung eines Bunthörnchens (*Sciurus variegatoides*) und der Virusnachweis mittels Hochdurchsatzsequenzierung führten zur Entdeckung des neuen Erregers. Öffentlichkeitsarbeit in Kombination mit dem Angebot einer kostenlosen Diagnostik am Friedrich-Loeffler-Institut (FLI) und entsprechenden Biosicherheitsmaßnahmen hat vermutlich zu einer Elimination des Erregers in deutschen Hörnchen-Haltungen geführt. Aktuelle Forschungsfragen beschäftigen sich mit der Aufklärung des Übertragungsweges, der Umweltstabilität sowie des geografischen Ursprungs des Erregers. Ein Überblick über humanpathogene Bornaviren findet sich in **Tab. 4**.

Frühsommer-Meningoenzephalitis-Virus – weiter auf dem Vormarsch

Das FSME-Virus gehört zur Gattung *Flavivirus* und wurde 1937 im fernen Osten der ehemaligen Sowjetunion entdeckt [72]. Dort waren insbesondere bei den Grenztruppen vermehrt Fälle einer schweren Enzephalitis (russische Frühjahr-Sommer-Enzephalitis, RSSE) aufgetreten. In den darauffolgenden Jahren wurden als Überträger die dort vorkommenden Schildzecken (Taigazecke, *Ixodes persulcatus*) identifiziert. Schon im Jahr 1931 beschrieb Schneider in Österreich ein Krankheitsbild („Schneider'sche Krankheit"), von dem wir heute annehmen können, dass es sich um die europäische Form der FSME handelte

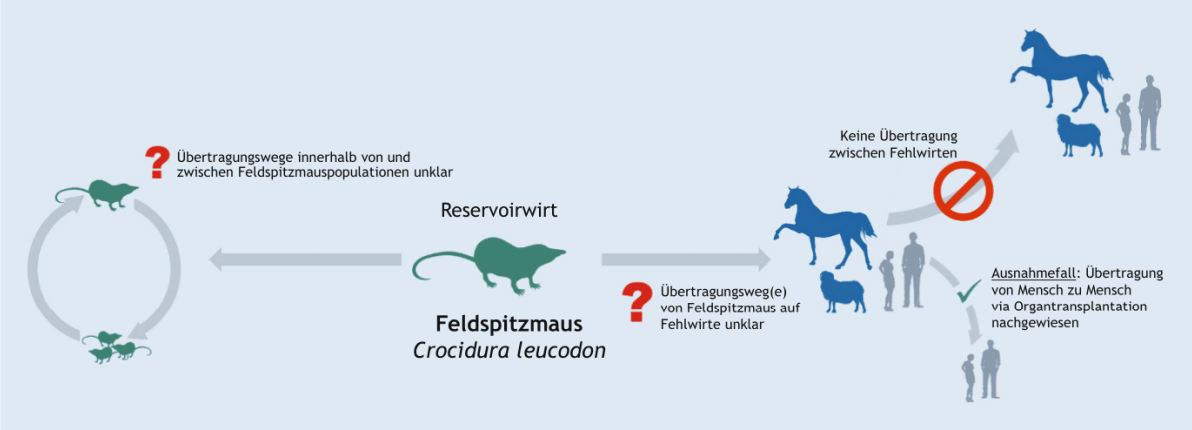

Abb. 4 ▲ Der Reservoirwirt des Borna Disease Virus 1 (BoDV-1) ist die Feldspitzmaus (*Crocidura leucodon*). Die Erkrankung bei Pferd und Schaf ist schon lange Zeit als „Borna'sche Krankheit" bekannt. Auch Neuweltkameliden wie Alpakas sind besonders empfänglich für das Virus. Erst vor wenigen Jahren ist jedoch zweifelsfrei gezeigt worden, dass BoDV-1 Enzephalitiden beim Menschen verursachen kann. Der genaue Übertragungsweg vom Reservoir auf den Menschen ist bisher nicht bekannt; eine Übertragung von Mensch zu Mensch durch Organtransplantation ist in einem Fall beschrieben. Auch die Situation im Reservoirwirt und die Stabilität des Erregers sind gegenwärtig noch wenig untersucht. Inwieweit bestimmte Umweltbedingungen eine Rolle bei der Übertragung spielen, ist ebenfalls unklar. Quelle: eigene Abbildung

[73]. In den 1940er-Jahren wurden ähnliche Krankheitsfälle auch in anderen Teilen Europas beschrieben und resultierten in der Entdeckung des FSME-Virus in der ehemaligen Tschechoslowakei im Jahr 1948. Dort wurden in den 1950er-Jahren große Epidemien beobachtet, die allerdings nicht durch Zeckenstiche, sondern durch kontaminierte Milch von großen und kleinen Wiederkäuern verursacht wurden. Heutzutage stellen Zeckenstiche, v. a. durch die Zeckenart *Ixodes ricinus* (Gemeiner Holzbock) den wichtigsten Übertragungsweg dar [74]. Es wird davon ausgegangen, dass es nur bei ca. 5–10 % der Stiche durch infizierte Zecken zu einer symptomatischen FSME kommt, wohingegen die alimentäre Übertragungsform fast immer klinisch in Erscheinung tritt [75, 76]. In den 1990er-Jahren zeigten genetische Untersuchungen, dass mindestens 3 (nach neueren Untersuchungen mindestens 5) unterschiedliche Subtypen des FSME-Virus existieren [77]. Neben dem europäischen Subtyp wurden auch ein sibirischer und ein fernöstlicher Subtyp beschrieben, die neben einer unterschiedlichen Ökologie auch unterschiedlich schwere Verlaufsformen der Infektion beim Menschen aufweisen können ([78]; ◘ **Tab. 1**).

Der natürliche Übertragungszyklus des FSME-Virus vollzieht sich zwischen Zecken als natürlichen Überträgern und verschiedenen Kleinsäugerwirten. Hierzu zählen in Europa v. a. Rötelmaus, Gelbhalsmaus (*Apodemus flavicollis*) und ggf. Spitzmäuse ([79]; ◘ **Abb. 5**). Die natürlichen Wirte entwickeln eine ausreichend hohe Virämie, so dass blutsaugende Zecken sich wieder infizieren können. Das sog. Co-Feeding stellt eine nichtvirämische Übertragungsform dar, bei der das Virus von einer infizierten Nymphe auf eine Larve übertragen wird, die in unmittelbarer Nachbarschaft zu dieser Nymphe Blut saugt. Dabei findet eine Übertragung statt, ohne dass das Wirtstier (i. d. R. ein Nagetier) infiziert oder gar virämisch ist. Größere Wildtiere, Haustiere und der Mensch sind Fehlwirte. Sie tragen nicht zur Zirkulation des Virus in der Natur bei. Dagegen scheiden Ziegen, Schafe und Kühe das Virus über die Milch aus. Der Verzehr dieser unbehandelten viruskontaminierten Milch kann zur Infektion führen [80]. Dieser Übertragungsweg war bis in die jüngste Vergangenheit hauptsächlich in Osteuropa bekannt und war dort für bis zu 17 % der Übertragungen des FSME-Virus verantwortlich [74]. In den letzten Jahren wurde die Übertragung durch Milch vereinzelt in Deutschland, Österreich und Frankreich beobachtet, mit teilweise mehr als 40 humanen Erkrankungsfällen bei einzelnen Ausbrüchen [76, 81, 82].

Das FSME-Virus kommt in großen Teilen Europas (exklusive Spanien, Portugal) und im Norden Asiens (Russland, Mittelasien, China, Japan) vor und wurde erstmals auch in Nordafrika nachgewiesen [83]. In Europa ist die FSME eine meldepflichtige Infektionskrankheit und es werden jährlich mehrere Tausend Erkrankungsfälle registriert. Zu den Ländern mit der höchsten Inzidenz in Europa zählen Schweden, Finnland, die baltischen Staaten, Tschechien, die Slowakei, Slowenien und Österreich [84].

In Deutschland werden jährlich zwischen 350 und 700 FSME-Fälle gemeldet [11]. Davon treten ca. 85 % in den beiden Bundesländern Bayern und Baden-Württemberg auf. Auch im Bundesland Sachsen ist in den letzten Jahren ein starker Anstieg der Erkrankungsfälle zu verzeichnen. Insgesamt ist wie auch in den benachbarten Ländern Österreich, Tschechische Republik und Schweiz ein deutlich zunehmender Trend an Erkrankungsfällen zu beobachten [83]. Aktuell gibt es keine wirksame und kausale Therapie der FSME – alle therapeutischen Maßnahmen beschränken sich auf die Behandlung der Symptome. Zur Prophy-

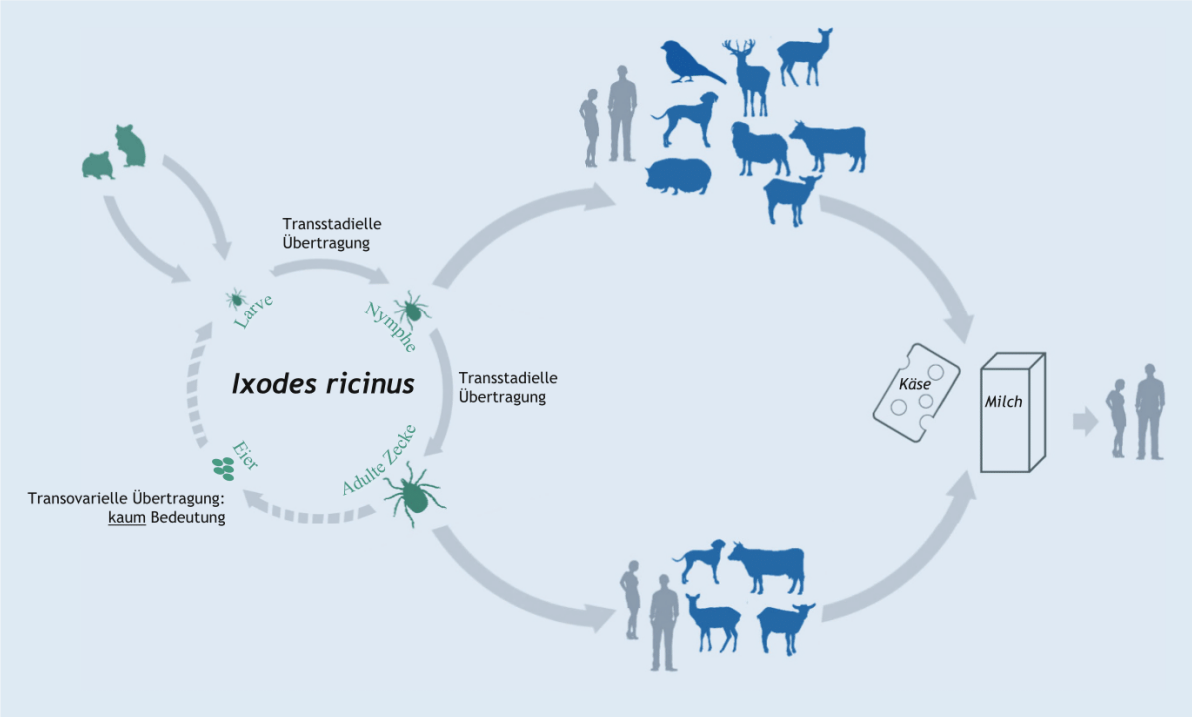

Abb. 5 ▲ Die Übertragung des Frühsommer-Meningoenzephalitis-(FSME-)Virus ist an den Zeckenvektor, vor allem den Gemeinen Holzbock (*Ixodes ricinus*), gebunden. Der Lebenszyklus der Zecke erfordert eine Blutmahlzeit in jedem Entwicklungsstadium nach dem Schlupf aus dem Ei. Die Larven saugen an Kleinsäugern, vor allem Nagetieren, die gleichzeitig auch Virusreservoir sind. Dort werden während der Blutmahlzeit die FSME-Viren aufgenommen und bleiben lebenslang in der Zecke. Nach der Häutung zur Nymphe kann diese die Infektion auf eine Vielzahl von Tieren und den Menschen übertragen. Ähnliches gilt für die weiblichen adulten Zecken. Die alimentäre Infektion über infektiöse Milch oder Rohmilchprodukte virämischer, infizierter Wiederkäuer ist ebenfalls dargestellt. In solchen Fällen kommt es meist zu einer Häufung von Erkrankungen durch den gemeinsamen Verzehr und zu einer hohen klinischen Manifestation. Der namensgebende „Frühsommer" wird immer mehr durch ein ganzjähriges Auftreten ersetzt, da durch die warmen Temperaturen die Zecken auch im Winter aktiv sind. Quelle: eigene Abbildung

laxe der FSME stehen 2 sehr wirksame und gut verträgliche Impfstoffe zur Verfügung. Die FSME-Impfung wird allen Personen empfohlen, die sich in den vom RKI ausgewiesenen Risiko-Landkreisen aufhalten und gegenüber Zecken exponiert sind [85].

Kenntnisse zur Ökologie des FSME-Virus in der Natur und zu seinem Übertragungszyklus stammen vor allem aus den 1950er- und 1960er-Jahren. Dabei zeigt sich zunehmend, dass viele der daraus resultierenden Konzepte neu überdacht werden müssen. Bisher ist der potenzielle Einfluss von Umweltfaktoren auf die Populationsdynamik der Wirte und Zecken und deren Durchseuchungsraten nur wenig verstanden. Insbesondere ist weitgehend unklar, inwieweit sich der Klimawandel auf den Übertragungszyklus des Virus, auf die Virulenz des Erregers und damit auf die Epidemiologie der Erkrankung beim Menschen auswirken wird [86]. Unverstanden sind bisher auch die Ursachen für die ungewöhnliche Epidemiologie in Deutschland (Süd vs. Nord) und den sich deutlich abzeichnenden ansteigenden Trend der Erkrankungsfälle in Mitteleuropa, darunter u. a. auch in Österreich, einem Land mit einer Impfquote von rund 85 %.

West-Nil-Virus – weiteres Arbovirus in Deutschland seit Kurzem etabliert

Das West-Nil-Virus (WNV) gehört wie das FSME-Virus zur Gattung *Flavivirus*. Es wurde erstmals 1937 in Nordwest-Uganda, im damaligen West-Nil-Distrikt, bei einer fieberhaft erkrankten Frau isoliert [87]. In Afrika war das Virus lange ohne große Bedeutung, da in Afrika andere fieberhafte Infektionen (z. B. Malaria) die Zahl der wenigen symptomatischen WNV-Infektionen bei Weitem überstieg. In Europa wurden erst 1962 Erkrankungsfälle bei Menschen und Pferden in der Camargue (Südfrankreich) und wenig später im Süden Portugals entdeckt [88], blieben aber weiter lokal begrenzt. In den 1990er-Jahren kam es zu einigen auch größeren Ausbruchsgeschehen in Rumänien, Russland, Italien und der Tschechischen Republik. Dem Virus gelang der Sprung in die neue Welt nach New York [89]; von dort aus hat es sich innerhalb zweier Jahrzehnte nach ganz Nord-, Mittel- und Südamerika ausgebreitet [90]. In Europa wird WNV mittlerweile in vielen südlichen Ländern nachgewiesen und ist

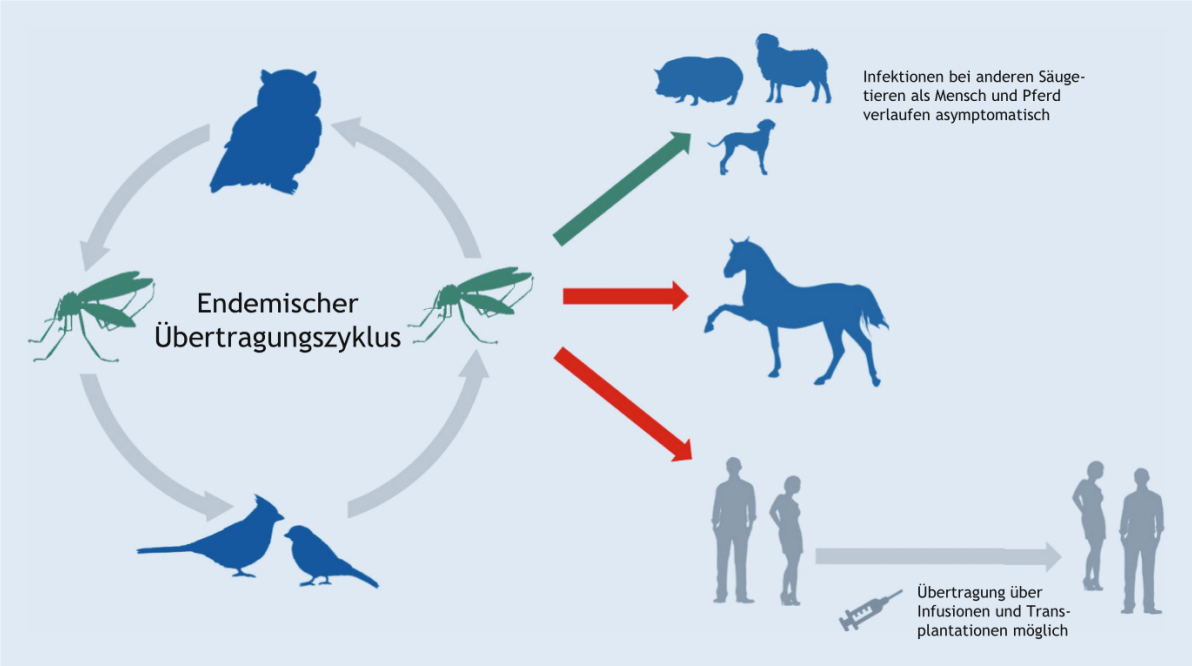

Abb. 6 ▲ Der endemische Übertragungszyklus des West-Nil-Virus (WNV) beinhaltet die Übertragung von bestimmten Mückenarten, meist aus dem Genus *Culex*, auf eine Vielzahl von unterschiedlichen Vogelarten. Zum späteren Sommer hin neigen die eigentlichen ornithophilen Stechmückenarten dazu, ihre Blutmahlzeit auch an Säugern zu nehmen und fungieren damit als sogenannte Brückenvektoren, die damit das Virus aus dem Vogelkreislauf auf die unterschiedlichen Säugetierspezies übertragen. Diese Infektion kann bei Haus- und Wildtieren ohne sichtbare Klinik erfolgen und nur zur Serokonversion führen (grüner Pfeil). Oder die Infektion führt, wie bei Mensch und Pferd bei einem bestimmten Prozentsatz zu Erkrankungen (roter Pfeil). Die Übertragung von WNV von Mensch zu Mensch kann auch durch Bluttransfusion und Organtransplantation erfolgen. Je wärmer die Jahre werden, desto schneller kann sich das Virus im endemischen Übertagungszyklus vermehren und desto früher im Jahr kommt es zu den ersten Fällen bei Mensch und Tier. Tiere könnten in einem One Health-Ansatz als Sentinel besser genutzt werden, um das Risiko humaner Infektionen geografisch und zeitlich besser einschätzen zu können. Quelle: eigene Abbildung

seit 2018 auch in Deutschland endemisch. Im ostdeutschen Tiefland wird es regelmäßig nachgewiesen [91, 92].

Antigenetisch gehört das Virus, wie das ökologisch und genetisch nahverwandte Usutu-Virus, zum japanischen Enzephalitis-Komplex der Gattung *Flavivirus*. Es sind mindestens 7 genetische Linien bekannt, wobei nur die Linien 1 und 2 Erkrankungen bei Mensch und Pferd verursachen können [93].

Das WNV ist ein Arbovirus, das in der Natur zwischen Stechmücken (Vektor) und Vögeln (Amplifikationswirte) zirkuliert (**Abb. 6**). Weibliche Stechmücken, die Wirbeltierproteine für ihre Eireifung benötigen, nehmen das Virus bei einer Blutmahlzeit von virämischen Vögeln auf und können es bei der nächsten Blutmahlzeit wieder auf andere Wirbeltierwirte übertragen. Dies sind in der Regel wiederum Vögel, so dass sich der natürliche Übertragungszyklus (enzootischer Zyklus) schließt [94]. Wenn Stechmücken sich nicht wirtsspezifisch ernähren, sondern als sog. Generalisten fungieren, können die Viren auf verschiedene Säugetiere übertragen werden. Wie in der **Abb. 6** dargestellt, können dies unterschiedliche Wildtiere (z. B. Wildschweine) oder Haustiere (Hunde, Schafe) sein, die serokonvertieren, in der Regel jedoch nicht klinisch erkranken. Anders ist dies bei Pferden und Menschen. Auch hier werden keine längeren Virämien mit hohen Viruslasten erzeugt, an denen sich naive Stechmücken infizieren könnten (daher Fehlwirt), dennoch kann es zu klinisch apparenten Infektionen kommen [95].

Nach maximal zweiwöchiger Inkubationszeit kommt es bei 10–20 % der infizierten Personen zu plötzlichem Fieber mit Schüttelfrost, Kopf- und Gliederschmerzen (**Tab. 1**). Diese grippeähnliche Erkrankung klingt in der Regel nach ca. einer Woche ab [96]. Nur bei etwa jedem 100. Infizierten kommt es zur Infektion des ZNS mit den unterschiedlichen klinischen Ausprägungen einer Meningoenzephalitis (West Nile Neuroinvasive Disease – WNND). Diese äußert sich je nach betroffenem Hirnareal eher motorisch mit schlaffen Lähmungen und Ataxien oder mit Bewusstseinsänderungen, Gedächtnisverlust oder Verwirrtheit. Die WNND ist eine schwere Erkrankung, für die keine Kausaltherapie zur Verfügung steht und die eine Letalität von etwa 10 % aufweist [96, 97]. Impfstoffe sind bisher nur für Pferde in Deutschland zugelassen und von der Ständigen Impfkommission Veterinärmedizin (StIKo Vet) des

FLI wird eine prophylaktische Impfung in den WNV-Verbreitungsgebieten empfohlen [98].

Seit dem ersten Auftreten im Jahr 2018 wurde das WNV in Deutschland regelmäßig bei Vögeln nachgewiesen [99]. Der erste humane Fall 2018 geht auf eine Infektion während der Sektion eines infizierten Vogels zurück; bei den nachfolgenden humanen Fällen wird zum einen eine Übertragung von Stechmücken, aber andererseits auch eine Übertragung im Rahmen von Blutspenden als sehr wahrscheinlich angesehen [14, 100]. Insgesamt sind mehrere Fälle von WNND in Deutschland, fast ausnahmslos bei älteren Menschen, diagnostiziert worden; letale Verläufe kamen vor [97, 100].

Derzeit ist nicht bekannt, ob es neben dem Alter und der Exposition weitere Risikofaktoren für ein West-Nil-Fieber oder eine WNND gibt. Das Wildvogel-Monitoring ist hilfreich, um frühzeitig in der Übertragungssaison das Zirkulieren von WNV zu erkennen [91, 101]. Und auch das in Deutschland durchgeführte Stechmücken-Monitoring trägt wesentlich zu unserem Verständnis bei, vor allem wo, wann, wie viele und welche Stechmückenarten Träger des Virus und damit mögliche Überträger sind [102]. Jedoch bleiben bei diesem Infektionsgeschehen noch sehr viele Fragen offen, die nur in einem One Health-Ansatz zufriedenstellend beantwortet werden können. Weitere Tierarten (**Abb. 6**) könnten als Sentineltiere, wie gegenwärtig noch Pferde und nach umfangreicher Durchimpfung der Pferdepopulation Wirtschaftsgeflügel in Freilandhaltung oder Zoovögel, helfen, das aktuelle Infektionsrisiko des Menschen besser zu beurteilen [103]. Die Frage, warum bei Mensch und Pferd nur ein bestimmter, zum Glück kleiner Anteil der Infizierten schwer erkrankt, impliziert bestimmte Prädispositionen. Deren Kenntnis könnte ggf. genutzt werden, diese Menschen besser zu schützen.

Zoonotische Influenza-A-Viren als potenzielle Gefahr

Zu den zoonotischen Influenza-A-Viren (IAV) gehören bestimmte aviäre Influenzaviren (AIV), insbesondere die asiatischen hochpathogenen AIV des Subtyps H5N1 und AIV des Subtyps H7N9 in China, aber auch einige der aviären H9N2-Viren [104]. In Deutschland sind bisher keine humanen Infektionen mit AIV berichtet worden. Anders verhält es sich bei der zweiten Gruppe, den Schweineinfluenzaviren (SIV). Ein bedeutendes Beispiel der jüngeren Zeit ist das pandemische H1N1-Virus von 2009 („Schweinegrippe"). Darüber hinaus kommt es immer wieder auch zu einzelnen Spillover-Infektionen bei Menschen in Deutschland mit im Schwein endemisch vorkommenden SIV der Subtypen H1 und H3, wobei es dann oft jüngere Personen betrifft und in der Regel ein Kontakt zu Schweinen bzw. Schweinehaltungen besteht. So konnten in Deutschland in den vergangenen 3 Jahren 2 solcher Infektionen erfasst und berichtet werden [105, 106]. Die klassischen SIV mischen sich zudem seit 2009 mit dem pandemischen H1N1 und es entstehen zahlreiche neue Reassortanten, die weiter beobachtet werden müssen [107].

Das zoonotische Potenzial der aktuell in Deutschland bei Wildvögeln zirkulierenden hochpathogenen AIV vom Subtyp H5N1 (HPAIV H5N1 clade 2.3.4.4B) ist immer noch als gering einzustufen, auch wenn es in Deutschland vereinzelte Übertragungen auf Karnivoren gegeben hat und weltweit bisher 7 Fälle beim Menschen berichtet wurden [108]. Hier ist es in jedem Fall notwendig, die Situation aufmerksam weiterzuverfolgen und insbesondere weitere Übertragungsfälle auf Säugetiere genau zu untersuchen. Dies ist insbesondere vor dem Hintergrund der Berichte von ersten „Säugetier-zu-Säugetier"-Übertragungen in einer Nerzfarm in Spanien [109] und eventuell auch bei Seelöwen in Peru von Bedeutung ([110]; siehe **Tab. 1**).

Fazit

Die transdisziplinäre und sektorenübergreifende Zusammenarbeit hat bei zoonotischen Erkrankungen bereits eine lange Tradition. Diese wird auch für Kleinsäuger-assoziierte Erreger durch das Netzwerk „Nagetier-übertragene Pathogene" [111] belegt. Eine intensive Zusammenarbeit von Human- und Veterinärmedizin hat in der Vergangenheit zur erstmaligen Aufdeckung der Ursache von VSBV-1-bedingten humanen Enzephalitis-Fällen beigetragen [71]. Die One Health-Herangehensweise, bei der dann auch die Bio- und Umweltwissenschaften einbezogen werden, hat zu einem deutlich verbesserten Verständnis in der Epidemiologie viraler Zoonosen geführt. Nur durch dieses Verständnis werden die nachhaltige Bekämpfung dieser Erkrankungen und der Schutz vor Ansteckung sowie die Erarbeitung und/oder Anwendung nachhaltiger Kontrollmaßnahmen ermöglicht. Insbesondere Untersuchungen in Deutschland zu Hantaviren, Bornaviren oder FSME-Viren belegen die gemeinsame und holistische Aufklärung der Ursachen von Erkrankungshäufungen wie auch von ökologischen Prozessen. Ein besonders hervorzuhebendes Beispiel sind parallele BoDV-1-Untersuchungen von Menschen, Reservoirwirten, Vektoren und der Umwelt in Maitenbeth, einer Gemeinde in Bayern [10].

Während die Aufklärung der ätiologischen Ursachen von potenziell zoonotischen Erkrankungen voranschreitet, z. B. bei Bornaviren (siehe [71]), hat die Entdeckung „neuer", d. h. bisher nicht bekannter Viren in Reservoirwirten zu einer erheblichen Wissenslücke bezüglich deren zoonotischen Potenzials geführt [24]. Andererseits trägt die Entdeckung bisher unbekannter Viren in den unterschiedlichen Reservoiren zu einer besseren Kenntnis der zirkulierenden Erreger bei, was eine wichtige Größe in der Prävention und Kontrolle von Infektionsgeschehen an der Mensch-Tier-Umwelt-Schnittstelle darstellt. Nichtsdestoweniger geht man gegenwärtig davon aus, dass nur ein Bruchteil der existierenden Viren bekannt ist [112]. Das betrifft beispielsweise auch Viren bei Spitzmäusen, wo erst in der jüngsten Vergangenheit eine „Virussuche" begonnen hat [113]. Neben dem Fokus „Zoonosen" sollten aber auch Erreger im Auge behalten werden, die bisher nur als Verursacher von Erkrankungen beim Tier erfasst worden sind und deren zoonotisches Potenzial noch unklar ist, wie z. B. beim Rustrelavirus [114–116].

Die One Health-basierten Untersuchungen berücksichtigen zunehmend

auch die Frage der Folgen des Klimawandels [12]. Natürlich konzentrieren sich diese One Health-Untersuchungen nicht nur auf virale Erreger, sondern beziehen auch alle anderen Erregergruppen, wie bakterielle Erreger, inkl. der Frage der Antibiotikaresistenzen, und Endoparasiten ein. Darüber hinaus wird zunehmend auch die Rolle des Mikrobioms und die Bedeutung von Co-Infektionen, inkl. solcher mit nicht zoonotischen Viren in Kleinsäugern, berücksichtigt, um ein ganzheitliches Bild der Gefährdung von Mensch und Tier zu generieren.

Die im Rahmen des One Health-Ansatzes gewonnenen Erkenntnisse werden zu einer ganzheitlichen Herangehensweise der Bekämpfung von Infektionskrankheiten und der Aufklärung der Bevölkerung beitragen. Gleiches gilt für die Kommunikation zwischen speziellen Risiko- und Fachgruppen in Humanmedizin, Veterinärmedizin, Epidemiologie und Biologie. Für die Ärztinnen und Ärzte des öffentlichen Gesundheitsdienstes wurden hierzu bereits mehrere Workshops im Rahmen der Jahrestagung ihres Bundesverbandes durchgeführt und die Erfahrungen daraus evaluiert [117].

Da für viele zoonotische Erreger, wie z. B. Hantaviren und HEV, für Menschen in Europa noch kein Impfstoff verfügbar ist und auch in absehbarer Zeit nicht zur Verfügung stehen wird, sind andere präventive Maßnahmen zur Verhinderung notwendig. Um die Gefährdung einer Erregerübertragung durch zum Beispiel Stechmücken zu reduzieren, sollten physikalische, biologische und chemische Verfahren der Mückenbekämpfung angewandt werden. Dazu sollten u. a. stehende, künstliche Gewässer (z. B. Regentonne) regelmäßig geleert werden, um den Stechmücken kein Bruthabitat zu bieten. Zur Vermeidung von Stichen sollte lange Kleidung getragen, Mückenschutzmittel aufgetragen und die Fenster der Schlafräume mit Mückengaze verschlossen werden [100]. Zur besseren Erkennung von humanen Fällen von Zoonosen sollten Ärztinnen und Ärzte entsprechend geschult werden, so dass die gezielte Diagnostik auch angefordert wird. Nur ein gezieltes Zusammenwirken von Ärztinnen und Ärzten der Veterinär- und Humanmedizin sowie des Öffentlichen Gesundheitsdienstes (ÖGD), der Veterinärämter mit den Expertenlaboren, der mit der Mückenbekämpfung betrauten Institutionen und der Umweltwissenschaftlerinnen und -wissenschaftler wird zukünftig eine bessere Überwachung von – in vielen Fällen potenziell tödlichen – Zoonosen ermöglichen. Im Sinne des One Health-Ansatzes wurde zum Beispiel das Merkblatt „Wie vermeide ich Hantavirus-Infektionen“ unter Einbeziehung human- und veterinärmedizinischer sowie Umwelt-Expertise gemeinsam durch Robert Koch-Institut, Friedrich-Loeffler-Institut, Bernhard Nocht-Institut und Julius Kühn-Institut erarbeitet.[1] Analog dazu wurde auch ein Merkblatt zur Vermeidung von Bornavirus-Infektionen erstellt.[2] Das Bundesinstitut für Risikobewertung (BfR) hat zudem Fragen und Antworten zur Hepatitis E-Virus-Übertragung mit Empfehlungen zum Schutz vor Infektionen zusammengestellt.[3]

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[1] https://www.rki.de/DE/Content/InfAZ/H/Hantavirus/Merkblatt_PDF.html [Zugriffsdatum: 30/03/2023].

[2] https://www.rki.de/DE/Content/InfAZ/B/Bornavirus/Merkblatt.pdf [Zugriffsdatum: 30/03/2023].

[3] https://www.bfr.bund.de/cm/343/fragen-und-antworten-zur-uebertragung-des-hepatitis-e-virus-durch-wild-und-hausschweine-und-daraus-gewonnene-lebensmittel.pdf [Zugriffsdatum: 30/03/2023].

Einhaltung ethischer Richtlinien

Interessenkonflikt. R.G. Ulrich, S. Drewes, V. Haring, J. Panajotov, M. Pfeffer, D. Rubbenstroth, J. Dreesman, M. Beer, G. Dobler, S. Knauf, R. Johne und M.M. Böhmer geben an, dass kein Interessenkonflikt besteht.

Für diesen Beitrag wurden von den Autor/-innen keine Studien an Menschen oder Tieren durchgeführt. Für die aufgeführten Studien gelten die jeweils dort angegebenen ethischen Richtlinien.

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2.5 Orthobornaviruses and their zoonotic potential

2.5.1 Genome structure of orthobornaviruses

The family *Bornaviridae* (order *Mononegavirales*) is divided into three genera: *Orthobornavirus*, *Carbovirus* and *Cultervirus* (Rubbenstroth et al., 2021). Carboviruses and culterviruses were so far only detected in reptiles and fish (Eshak et al., 2023), whereas orthobornaviruses were detected in multiple mammalian, avian and snake species (**Figure 6**).

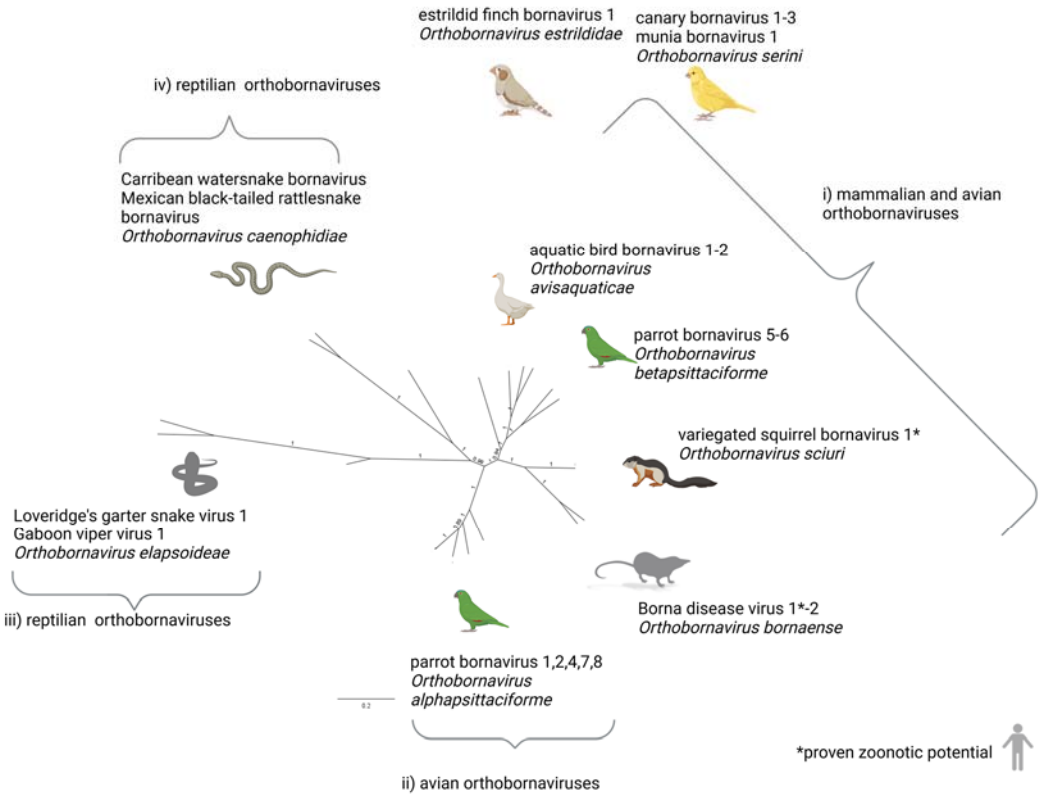


Figure 6 Schematic organization of the genus *Orthobornavirus.* The definition of different orthobornaviruses is based on Pairwise Sequence Comparison (PASC, cut-off of coding-complete genome sequences: 72-75% (Kuhn et al., 2015)) in combination with biological features. Four groups were established comprising (i) mammalian and avian orthobornaviruses, (ii) avian orthobornaviruses, (iii and iv) reptilian orthobornaviruses (Rubbenstroth et al., 2021). The phylogenetic tree was calculated in Geneious version 2021.0 (Biomatters, available from https://www.geneious.com). Created with BioRender.com agreement number: YE27TRDZ0J.

Orthobornaviruses are enveloped viruses with a diameter of 90-130 nm (Rubbenstroth et al., 2021) (**Figure 7A**). The single-stranded, non-segmented, negative-sense RNA genome ((-) ssRNA) consists of approximately 9 kilobases (kb) with six partially overlapping open reading frames (ORFs) on its complementary positive-stranded RNA (cRNA) encoding for the nucleoprotein (N), accessory protein (X), phosphoprotein (P), matrix protein (M), surface glycoprotein (G) and large protein (L), including the RdRp (Briese et al., 1994; Rubbenstroth et al., 2021) (**Figure 7B**). Replication of orthobornaviruses

takes place in the nucleus of the host cell and uses the cellular splicing machinery for alternative splicing of viral messenger RNA (mRNA) (Briese et al., 1992; Schneider et al., 1994; Tomonaga et al., 2002).

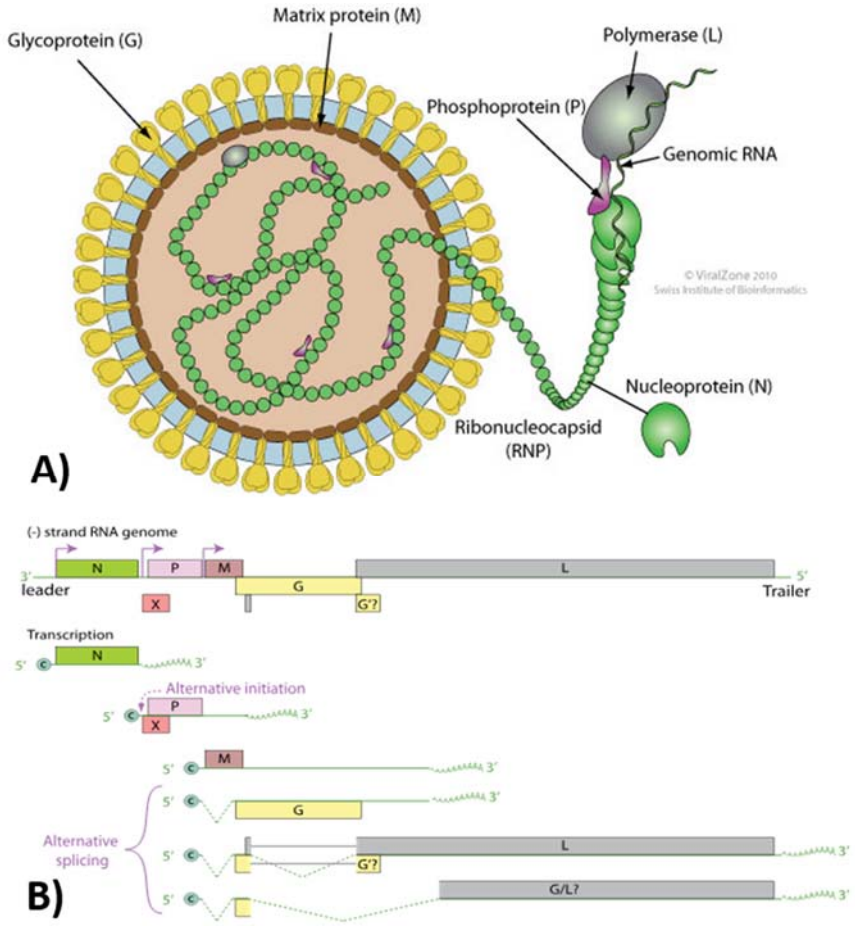


Figure 7 A) Virion structure of Borna disease virus 1 (BoDV-1, species *Orthobornavirus bornaense*); B) Genome organization of orthobornaviruses: reference sequence BoDV-1 NC_001607. The graphics are obtained from ViralZone, SIB Swiss Institute of Bioinformatic, licensed under a Creative Commons Attribution 4.0 International License (CC-BY).

Interestingly, so-called endogenous bornavirus-like (EBL) genetic elements resembling parts of the N, M, G and L genes were identified in the genomes of multiple species, including rodents, humans and non-human primates, indicating a long evolutionary history of bornaviruses. For non-human primates the integration of the detected EBLs has been hypothesized to have happened > 40 Myr ago (Horie et al., 2010; Belyi et al., 2010; Horie et al., 2013; Horie 2017; Kawasaki et al., 2021). An N-encoding EBL (itEBLN-1) was identified in the genome of a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), which according to phylogenetic analysis belongs to the genus *Orthobornavirus*, suggesting a more recent integration (~ 0.3 Myr) (Fujino et al., 2014; Suzuki et al., 2014).

2.5.2 Epidemiology, reservoir and transmission

Mammalian orthobornaviruses, namely Borna disease virus 1 and 2 (BoDV-1, BoDV-2; both species *Orthobornavirus bornaense*) and VSBV-1 (species *Orthobornavirus sciuri*), have a broad cell and organ tropism in their respective reservoir and are shed via urine and faeces (Nobach et al., 2015; Schlottau et al., 2017a), but are strictly neurotropic in accidental dead-end hosts (e.g. humans, sheep, New World camelids, and horses) (Caplazi et al., 1999; Hoffmann et al., 2015; Weissenböck et al., 2017; Niller et al., 2020; Nobach et al., 2020; Malbon et al., 2022; Fürstenau et al., 2023). Fatal encephalitis is induced in dead-end hosts by T-lymphocyte mediated immunopathogenesis (Bilzer and Stitz 1994) and symptoms are locomotor dysfunction (e.g. gait ataxia) and sensory dysfunction (e.g. somnolence, paralysis of facial nerve) leading to paralysis and death (Richt and Rott 2001). *Crocidura leucodon* was identified as reservoir for BoDV-1 (Hilbe et al., 2006) and zoonotic VSBV-1 was detected in captive exotic squirrel species (mostly variegated squirrels (*Sciurus variegatoides*) and Prevost's squirrels (*Callosciurus prevostii*)) in Germany, the Netherlands and Croatia (Hoffmann et al., 2015; Schlottau et al., 2017a; Schlottau et al., 2017b). BoDV-2 has been isolated from a diseased horse in Austria, but no natural reservoir has been identified yet (Weissenböck et al., 1998b). The transmission routes between the reservoir species and the dead-end hosts are still under study (**Figure 8**), but a possible rhinogenic infection with neuronal spread has been demonstrated in experimentally inoculated Lewis rats (Carbone et al., 1987; Kupke et al., 2019; Sauder and Staeheli 2003).

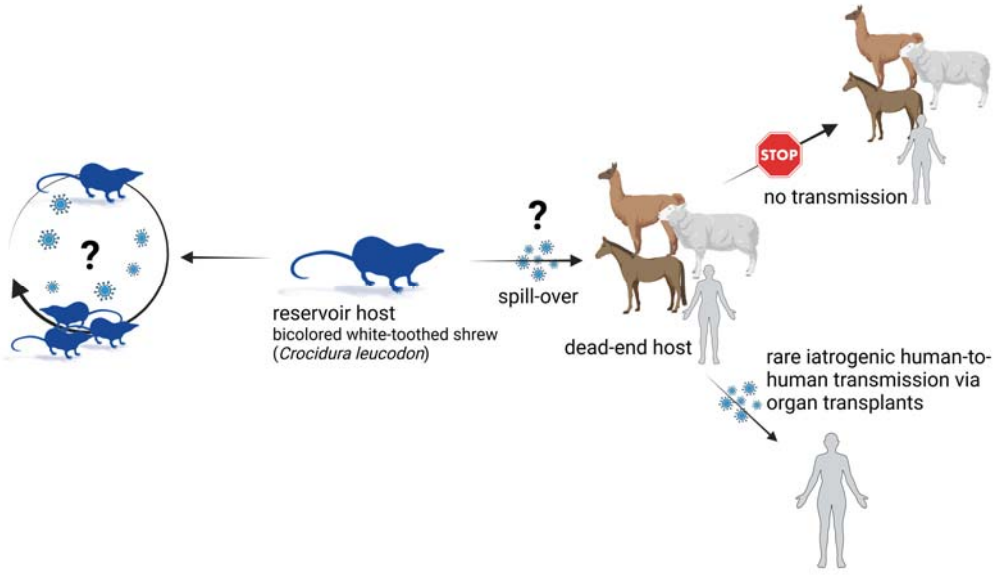


Figure 8 Epidemiology of BoDV-1 infections. The bicolored white-toothed shrew (*Crocidura leucodon*) has been identified as natural reservoir for Borna disease virus 1 (BoDV-1, species *Orthobornavirus bornaense*), which causes (lethal) encephalitis in domestic animals (mainly horses, New World camelids, sheep) and in humans. Transmission routes from the reservoir to the dead-end host remain unclear and the virus is most likely not further shed by the dead-end host. The zoonotic potential of BoDV-1 was revealed in an organ transplant cluster demonstrating potential, but rare iatrogenic human-to-human infection. Questions on virus persistence in the reservoir population, transmission between the hosts and the environmental tenacity remain still unanswered. Created with BioRender.com agreement number: DV27MKKE5X.

The endemic area of BoDV-1 is restricted to small regions in Switzerland, the Principality of Liechtenstein, Austria, and southern and eastern Germany (Dürrwald et al., 2006; Dürrwald et al., 2014a; Rubbenstroth et al., 2019) (**Figure 9 A**). Its known northern border was recently extended by the detection of BoDV-1 in an alpaca herd in north-eastern Germany (Schulze et al., 2020). Why the virus is restricted to such a defined region, while the distribution of the reservoir is many folds wider, remains puzzling. Phylogenetic relationships of BoDV-1 sequences have shown a strong association to geographic areas. This led to the establishment of five phylogeographic clusters containing sequences from the same region disregarding its host species (Kolodziejek et al., 2005) (**Figure 9 B**).

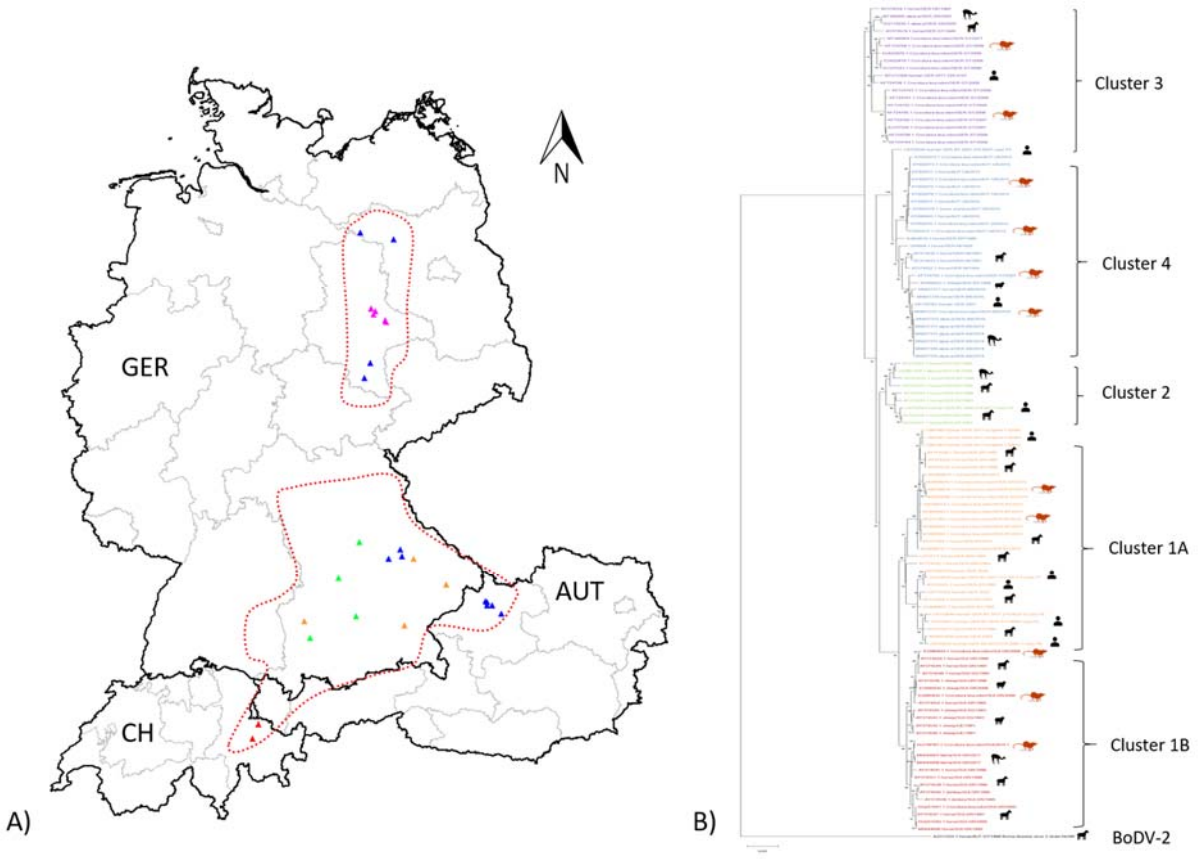


Figure 9 A) Map of the endemic area of BoDV-1. The currently known endemic area is marked in red and triangles represent shrew-derived BoDV-1 sequences indicating the geographic distribution of the respective phylogenetic cluster. GER: Germany, CH: Switzerland, AUT: Austria; **B) Maximum-Likelihood phylogenetic tree** of a 1,824 nucleotides long 5'-region spanning the N, X and P genes of BoDV-1 sequences generated from shrews, humans and domestic animals available on GenBank was conducted on MEGA X applying the Jukes-Cantor model and 1000 bootstrap replicates and demonstrates the five phylogeographic clusters established by Kolodziejek et al., (2005).

2.5.3 Zoonotic potential of BoDV-1

Since the identification of BoDV-1 as causative agent of the fatal encephalitis of two organ recipients (Schlottau et al., 2018; Korn et al., 2018) and thus proof of its zoonotic potential, more than 40 additional human cases have been identified, including retrospective cases dating back to 1996 (Coras et al., 2019; Rubbenstroth et al., 2019; Niller et al., 2020; Eisermann et al., 2021; Tappe et al., 2021; Rauch et al., 2022; Frank et al., 2022; Grosse et al., 2023). Human cases of BoDV-1 infections have been carefully evaluated to describe detailed clinical and epidemiological patterns of infection (Pörtner et al., 2023; Eisermann et al., 2021). Viral RNA can be detected in brain tissue, for *intra vitam* diagnostics in brain biopsies or sometimes in CSF (Neumann et al., 2023; Allartz et al., 2023), with diagnostic quantitative real-time reverse transcription polymerase chain reactions (RT-qPCRs) (Hoffmann et al., 2015; Schlottau et al., 2018; Sigrist et al., 2021; Allartz et al., 2023). For serum and CSF samples, detection of bornavirus antibodies with immunofluorescence antibody test (IFAT) is well established (Eisermann et al., 2021; Allartz et al., 2023). Seroconversion has been observed only during the course of disease (> 12 days after hospital admission) (Grosse et al., 2023; Allartz et al., 2023). Sero-epidemiological studies have resulted in very low detection of seropositive, non-symptomatic humans with only one sero-positive veterinarian, who`s future fate could not be followed due to anonymization of the study (Tappe et al., 2019; Bauswein et al., 2023). So far, the majority of cases succumbed to death despite off-label use of antivirals, ribavirin and favipiravir have proven to be effective *in-vitro* and *in-vivo* (Mizutani et al., 1998; Lee et al., 2008), and immunosuppressive treatment (Grosse et al., 2023), as no adequate treatment has been established. Early diagnosis may considerably increase the chances of success. Awareness of BoDV-1 as etiological cause for encephalitis by first opinion physicians is essential for early diagnosis, therefore a review article on the current state of knowledge on human BoDV-1 infection was published and is included in this thesis. Figures and tables are numbered according to the published chapter and references are presented in the journal style and do not appear in the reference section.

Review 2: Selten, aber tödlich: Bornavirus-Enzephalitis

Selten, aber tödlich: Bornavirus-Enzephalitis

Böhmer, Merle M.; **Haring, Viola;** Rubbenstroth, Dennis; Bauswein, Markus; Tappe, Dennis; Sternjakob, Anna; Pörtner, Kirsten; Frank, Christina; Wunderlich, Silke; Zimmer, Claus; Angstwurm, Klemens; Wiesinger, Isabel; Herden, Christiane; Beer, Martin; Schmidt, Barbara; Ulrich, Rainer G.

Bayerisches Ärzteblatt 9

2022

Quelle: Bayerisches Ärzteblatt 9/2022, Seite 434 ff

Varia

Selten, aber tödlich: Bornavirus-Enzephalitis

Das Borna Disease Virus-1 ist seit 2018 als seltener Erreger von fulminanten, meist letal verlaufenden Enzephalitiden beschrieben, mit einem Schwerpunkt in Bayern. Da die Erkrankung beim Menschen noch weitgehend unbekannt ist, wird die Diagnostik vermutlich zu selten angestrebt und Fälle bleiben undiagnostiziert.

Die Feldspitzmaus (Crocidura leucodon – Abbildung 1A) gehört zu den Insektenfressern, erreicht eine Körperlänge von 6 bis 8 cm und ist in Bayern weit verbreitet. Sie lässt sich morphologisch sehr gut von Wühlmäusen, zum Beispiel der Rötelmaus (Clethrionomys glareolus – Abbildung 1B) und echten Mäusen, wie der Gelbhalsmaus (Apodemus flavicollis – Abbildung 1C) unterscheiden. Die Unterscheidung von anderen Spitzmäusen der Gattung Crocidura (zum Beispiel Haus- oder Gartenspitzmaus) ist für Laien hingegen fast unmöglich. Die Abbildungen wurden freundlicher Weise von Dr. Henning Vierhaus und Ulrike M. Rosenfeld zur Verfügung gestellt.

Hintergrund

Bereits seit mehr als 250 Jahren ist die Bornasche Krankheit bei Säugetieren mit dem klinischen Bild einer schweren Enzephalitis bekannt. Im Jahr 2018 wurde ihr Erreger – das „Borna disease virus-1" (BoDV-1) aus der Familie der „Bornaviridae" – erstmals als Auslöser schwerer Enzephalitiden beim Menschen identifiziert [1, 2].

Zunächst wurde BoDV-1 bei einem Cluster von drei Organempfängern mit Enzephalitis nachgewiesen, die Organe desselben Spenders erhalten hatten [3]. Seitdem wurden weitere BoDV-1-bedingte Enzephalitiden diagnostiziert, ohne Zusammenhang mit einer Organtransplantation, darunter auch retrospektiv nachgewiesene Fälle [3-14]. Aktuell (Stand Juli 2022) liegt die Anzahl publizierter und/oder dem Robert Koch-Institut (RKI) gemeldeter Fälle von Enzephalitiden mit Direktnachweis von BoDV-1 bei über 40 (Erkrankungsjahre 1992-2022).

Das zurzeit bekannte Virusreservoir von BoDV-1 ist die Feldspitzmaus („Crocidura leucodon") [15, 16, 17] (Abbildung 1A). Sie scheidet das Virus über Kot, Urin, Speichel oder die Haut aus, ohne selbst zu erkranken [18, 19]. Als Fehlwirte sind besonders Pferde, Schafe, Alpakas und selten andere Tierarten sowie der Mensch empfänglich und erkranken mit schweren neurologischen Symptomen [20-26]. Bei Fehlwirten bleibt das Virus fast ausschließlich auf das Nervensystem beschränkt, weshalb sie es nach aktuellem Kenntnisstand auf natürlichem Weg nicht ausscheiden.

Epidemiologie

Jährlich werden dem RKI nur zwei bis sieben akute BoDV-1-Enzephalitiden gemeldet, die Erkrankung ist insgesamt sehr selten. Ein Großteil der Fälle tritt in Bayern auf (Abbildung 2). Neben Bayern umfassen die Endemiegebiete – definiert vor allem durch das Vorkommen der Infektion bei Tieren – vorwiegend die östliche Hälfte Deutschlands sowie Teile der Schweiz, Liechtensteins und Österreichs [1, 7]. Beim Auftreten von BoDV-1-Enzephalitiden außerhalb der bekannten Endemiegebiete sollte eine Infektion in einem Endemiegebiet in Betracht gezogen werden. Ferner ist möglich, dass Endemiegebiete nicht vollständig bekannt sind. BoDV-1-Infektionen treten in allen Altersstufen auf, wobei sehr junge Kinder unter sechs Jahren bisher nicht unter den bekannten Fällen zu finden sind. Bei der Mehrzahl der Fälle ist keine Beeinträchtigung des Immunsystems bekannt. Männliche und weibliche Personen waren etwa in gleichem Maße von BoDV-1-Infektionen betroffen. Alle bisher bekannten natürlich infizierten Personen wohnten in sehr ländlichen Regionen. Wegen der hohen Übereinstimmung von BoDV-1-Sequenzen bei Patientinnen und Patienten mit jenen von Reservoir- und Fehlwirten in der jeweiligen Region, ist davon auszugehen, dass sich die meisten Patienten nahe ihres Wohnortes infizierten [7].

Bei allen bekannten Fällen trat im Verlauf eine Enzephalitis auf. Mehrere wissenschaftliche Studien ergaben keinen Anhalt für asymptomatische oder oligosymptomatische Verläufe [5, 27]. Für die kontrovers diskutierte Assoziation von BoDV-1

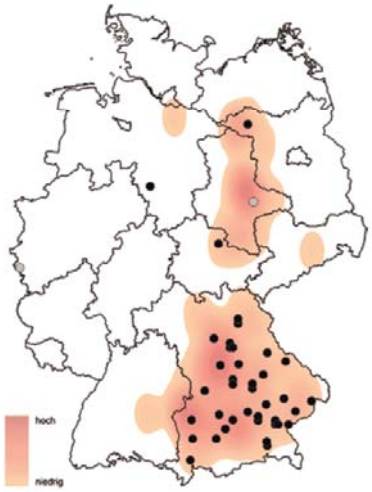
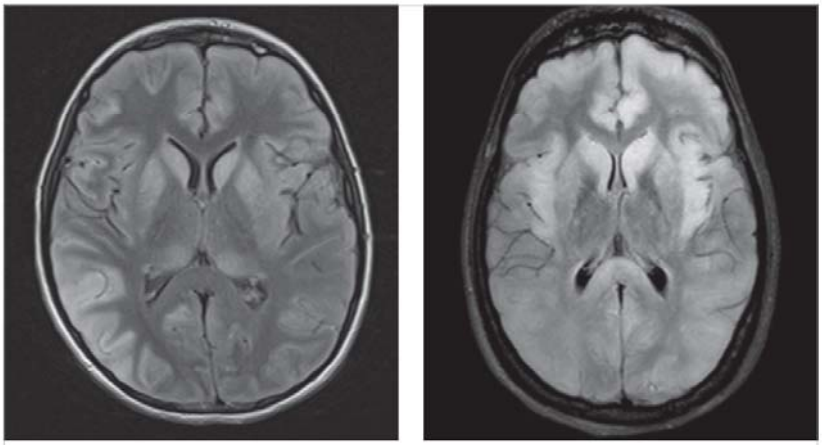


Abbildung 2: Gemeldete und publizierte humane BoDV-1-Infektionen mit bekanntem Wohnsitz (n = 42, 1992-2022; Stand: Juli 2022, Lokalisation nach Landkreis des Wohnorts). Fälle mit direktem Erregernachweis (schwarze Kreise) sind aus Bayern, Brandenburg, Thüringen und Niedersachsen beschrieben. Zudem gibt es je einen wahrscheinlichen, serologisch bestätigten Fall mit der entsprechenden Klinik der Enzephalitis aus Nordrhein-Westfalen und Sachsen-Anhalt (graue Kreise). Die drei iatrogen infizierten Fälle des Transplantationsclusters sind nicht dargestellt. Die orange schattierten Flächen markieren das bisher bekannte Endemiegebiet basierend auf Sequenz-validierten BoDV-1-Infektionen bei Spitzmäusen, Haussäugetieren und Menschen.



Patient 1
Bildquelle: Institut für Neuroradiologie, medbo-Bezirksklinikum, Regensburg

Kranielle MRT (axiale FLAIR) 21 Tage nach Symptombeginn.

Symmetrische Signalanhebungen insulär, Caput nuclei caudati und Thalamus sowie bifrontal und rechts parietal.

Patient 2
Bildquelle: Abteilung für Neuroradiologie, Klinikum rechts der Isar, Technische Universität München

Kranielle MRT (axiale FLAIR) 35 Tage nach Symptombeginn.

Symmetrische Signalanhebungen insulär, Caput nuclei caudati, in den Basalganglien, hinterer Balken sowie bifrontal und parietal.

Abbildung 3: MRT-Aufnahme von zwei Patienten mit BoDV-1-Enzephalitis. Charakteristisch sind die T2-hyperintensen Veränderungen v. a. der Stammganglien und in der Inselregion.

mit psychiatrischen Erkrankungen existieren keine belastbaren Daten [28].

Übertragungswege

Bisher ist unklar, wie das Virus vom Reservoirwirt auf den Menschen und andere Fehlwirte übertragen wird. Neben einer Übertragung durch direkten Kontakt zu Feldspitzmäusen ist eine indirekte Infektion durch virushaltige Ausscheidungen der Feldspitzmaus denkbar. So ist das Einatmen kontaminierten Staubs als Übertragungsweg vorstellbar. Auch könnte BoDV-1 über verunreinigte Lebensmittel oder Wasser aufgenommen werden oder durch Kontakt kontaminierter Erde mit Schleimhäuten. Ebenfalls könnte eine Übertragung über Haustiere erfolgen, die als passive „Bindeglieder" fungieren (zum Beispiel Katzen, die noch infektiöse Geweberückstände gefangener Spitzmäuse an Maul oder Pfoten aufweisen könnten) [7]. Bei keinem der bekannten Fehlwirte wurde bisher eine Erreger-Ausscheidung oder Weiterverbreitung nachgewiesen. Die BoDV-1-Übertragung von Mensch zu Mensch über transplantierte Organe stellt schon aufgrund der Seltenheit der Infektion einen extrem ungewöhnlichen Übertragungsweg dar.

Klinische Symptomatik

Das klinische Bild der BoDV-1-Enzephalitis ist anhand der bisherigen Fälle beschrieben [3-14]. Es wird angenommen, dass die Inkubationszeit – ähnlich wie bei Haussäugetieren – einen Zeitraum von wenigen Wochen bis einigen Monaten umfasst [7]. Bei den (immunsupprimierten) Organempfängern des Transplantationsclusters betrug die Inkubationszeit etwa drei Monate.

Bei den bekannten Fällen war der Krankheitsbeginn zumeist durch eine kurze Phase mit unspezifischer, grippaler Symptomatik gekennzeichnet [6-8]. Bei einigen wenigen Krankheitsfällen zeigten sich initial Symptome einer Beteiligung des peripheren Nervensystems, die zunächst zur Verdachtsdiagnose eines Guillain-Barré- oder Miller-Fisher-Syndroms oder einer Autoimmunenzephalitis führten [10, 12]. Im weiteren kurzen Verlauf entwickelten alle Patienten eine fulminante, über wenige Wochen rasch progrediente

Enzephalitis mit verschiedenen neurologischen Symptomen (darunter epileptische Anfälle, Ataxie, Myoklonien, Paresen, Halluzinationen), Bewusstseinsstörung bis hin zum tiefen Koma mit Ausfall der Hirnstammreflexe und schließlich in den meisten Fällen Tod [5, 8-11, 13, 14].

Labor und Bildgebung

Der Liquor zeigte bei Erstvorstellung meist ein unauffälliges oder unspezifisches Bild; eine Zellzahlerhöhung fehlte zum Teil sowohl in der Frühphase, aber auch über den gesamten Krankheitsverlauf. Im Verlauf zeigten sich eine mäßige Pleozytose, eine ausgeprägte Blut-Liquor-Schrankenstörung mit intrathekaler Immunglobulin-Synthese sowie einer Laktaterhöhung [8, 29]; ein auch im Verlauf vollständig unauffälliger Liquor scheint ungewöhnlich.

Bildmorphologisch zeigten BoDV-1-Erkrankte sowohl im frühen als auch im späten Krankheitsstadium ein charakteristisches Muster im MRT, teilweise blieb es allerdings auch in der schweren Krankheitsphase noch lange unauffällig. Typische Veränderungen waren symmetrische T2-Hyperintensitäten des Caput nuclei caudati, der Inselregion und des limbischen Systems häufig mit Aussparung des Okzipitallappens und des Kleinhirns (vgl. Abbildung 3) [11]. Neuropathologisch war eine nicht eitrige sklerosierende Panenzephalomyelitis typisch [10], die durch diffuse parenchymatöse lymphohistiozytäre Infiltration sowie starke Mikroglia- und Astrozytenaktivierung gekennzeichnet war [10, 14].

Diagnostik

Neben der Bildgebung und dem klinischen Bild einer schweren Enzephalitis ist die Untermauerung der Verdachtsdiagnose intra vitam serologisch aus Serum und/oder Liquor durch den Antikörpernachweis und den Anstieg der Antikörpertiter im Verlauf mittels Immunfluoreszenz-Antikörpertest, Immunoblot oder ELISA möglich. Aufgrund der Gefahr falsch positiver serologischer Befunde bedarf der Antikörpernachweis einer Bestätigung durch den direkten Virusnachweis [5]. Der Nachweis viraler RNA im Liquor mittels quantitativer Reverse-Transkription-Polymerase-Kettenreaktion (RT-qPCR) gelingt aufgrund der niedrigen RNA-Konzentrationen jedoch nicht in jedem Fall. Sehr sicher ist der Nachweis viraler RNA oder Virusantigen in bioptisch oder autoptisch entnommenem ZNS-Gewebe mittels RT-qPCR, in situ-Hybridisierung oder Immunhistochemie. Schleimhauttupfer, Blut oder Gewebe peripherer Organe eignen sich aufgrund des strikten Neurotropismus nicht für den Bornavirus-Nachweis. Für andere Nachweisverfahren, wie Bornavirus-Antigen im Blut sowie „zirkulierende Immunkomplexe" (CIC) mittels ELISA, fehlen wissenschaftliche Belege für die Anwendung zur Diagnostik einer BoDV-1-Enzephalitis. Es wird daher dringend davon abgeraten, sie zur Diagnostik zu nutzen (vgl. [30]).

Seit 1. März 2020 besteht eine Labormeldepflicht nach § 7 Infektionsschutzgesetz (IfSG) für den direkten Erregernachweis von humanpathogenen Bornaviren (bekannt: BoDV-1 und das bei exotischen Hörnchen in Deutschland gefundene variegated squirrel bornavirus 1, VSBV-1). Rein serologische Befunde können mit Blick auf eine schwerwiegende Gefahr für die Allgemeinheit (gemäß § 6 Abs. 1 Nr. 5 bzw. § 7 Abs. 2 IfSG) gemeldet werden. Eine BoDV-1-Diagnostik steht am Bernhard-Nocht-Institut für Tropenmedizin Hamburg sowie u. a. an bayerischen Universitätskliniken (u. a. Erlangen, München, Regensburg) zur Verfügung. Auskunft zum Nachweis beim Tier gibt das Nationale Referenzlabor für Bornavirus-Infektionen der Tiere am Friedrich-Loeffler-Institut (Kontaktadressen s. Anhang Literaturverzeichnis).

Indikationen für Bornavirus-Diagnostik

- Generell bei Personen mit schwerer (Meningo-)Enzephalitis unklarer Genese, insbesondere, wenn ein oder mehrere der folgenden Kriterien vorliegen:
  - Ländlicher Wohnort oder Aufenthalt im Endemiegebiet
  - Rasche Befundverschlechterung innerhalb von Tagen
  - Schwere EEG-Veränderung in den ersten Erkrankungstagen
  - Vorliegen eines charakteristischen MRT-Befunds mit symmetrischen T2-Hyperintensitäten von Basalganglien, der Insel und des limbischen Systems
  - Fälle von BoDV-1-Enzephalitis im gleichen Wohnort in der Vergangenheit
  - Kontakt zu Spitzmäusen oder deren Ausscheidungen/Körperflüssigkeiten
  - Erhalt eines Spenderorgans
  - Aufgrund überlappender Endemiegebiete: V. a. Frühsommer-Meningoenzephalitis (FSME) in Fällen, bei denen keine FSME-Virus-Infektion nachgewiesen wurde
  - Passender Liquorbefund (entzündliches Liquorsyndrom mit milder Pleozytose (im zweistelligen bis mittleren dreistelligen Bereich), Schrankenstörung, intrathekaler Immunglobulin-Synthese, Laktaterhöhung (bis zu 5fach)
- Bei Personen, die Kontakt zu Spitzmäusen oder deren Ausscheidungen/Körperflüssigkeiten hatten und bei denen nach einigen Wochen oder Monaten Symptome eines grippalen Infektes und zusätzlich neurologische Symptome neu auftreten.
- Differenzialdiagnosen aufgrund des bildgebenden Befundes und/oder des klinischen Verlaufs sowie nach Reiseanamnese:
  - Andere Virusenzephalitiden (v. a. FSME, HSV-1, Tollwut, Japanische Enzephalitis, Dengue-Fieber, West-Nil-Fieber, progressive multifokale Leukenzephalopathie)
  - Autoimmunenzephalitiden
  - Sporadische Creutzfeldt-Jakob-Krankheit

Tabelle 1: Hilfestellung für das Einleiten einer Bornavirus-Diagnostik

Therapie und Prophylaxe

Eine kurative Therapie der BoDV-1-Enzephalitis ist nicht etabliert. In-vitro haben sich die Virostatika Ribavirin und Favipiravir als wirksam gegen BoDV-1 erwiesen, beide sind jedoch nicht für die BoDV-1-Therapie zugelassen. Favipiravir hat in Europa keine Zulassung, wird aber in verschiedenen Apotheken aktuell noch bevorratet. Eine Beratung zur Anwendung und zum Bezug von Favipiravir kann jederzeit über die Behandlungszentren des STAKOB (www.stakob.de) erfolgen. Die Anwendung ist rein experimentell im Rahmen eines individuellen Heilversuchs möglich, die Dosierung und Länge der Therapie sind unklar, die Wirksamkeit ist nicht abschließend belegt. Eine (ggf. zusätzliche) immunsuppressive Therapie kann unter Umständen durch die Hemmung der T-Zell-vermittelten Immunpathogenese den Krankheitsverlauf verzögern. In einzelnen Fällen hat eine Therapie mit Ribavirin und/oder Favipiravir und/oder Immunsuppression den Verlauf der Erkrankung verzögert, eine durchgreifende Verbesserung der Klinik durch die Therapie ist bis jetzt nicht bekannt. Auch für ggf. zukünftig spezifischere Therapien erscheint

eine möglichst frühzeitige Diagnosestellung und rasche Einleitung der Therapie essenziell.

Ein Impfstoff gegen Bornavirus-Infektionen steht bislang nicht zur Verfügung. Da die Übertragungswege unbekannt sind und die Erkrankung auf einer T-Zell-vermittelten Immunpathogenese beruht, ist es schwierig, Empfehlungen zur Prävention oder einer etwaigen Postexpositions-Prophylaxe abzugeben. In jeden Fall sollte der Kontakt zu Spitzmäusen und deren Ausscheidungen dringend vermieden werden (vgl. RKI-Merkblatt, Link s. Anhang Literaturverzeichnis).

Wichtig für Ärztinnen und Ärzte in Bayern

Auch wenn vermutlich eine Reihe von BoDV-1-Infektionen undiagnostiziert bleibt, handelt es sich bei der BoDV-1-Enzephalitis dennoch um eine äußerst seltene Erkrankung. Da die Humanpathogenität zudem erst seit 2018 nachgewiesen ist, kann davon ausgegangen werden, dass das Krankheitsbild bei den behandelnden Ärzten – auch in Bayern – noch nicht breit bekannt ist. Eine frühzeitige Diagnosestellung aber ist essenziell, um etwaige Therapieversuche zu initiieren. In Tabelle 1 werden Hilfestellungen für das Einleiten einer Bornavirus-Diagnostik gegeben.

Wo finden Sie weitere Informationen und Beratung?

www.lgl.bayern.de/gesundheit/infektionsschutz/infektionskrankheiten_a_z/borna/index.htm

Das Literaturverzeichnis und weitere Informationen zum Thema können im Internet unter www.bayerisches-aerzteblatt.de (Aktuelles Heft) abgerufen werden.

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III. Objectives

White-toothed shrews are small elusive animals and show synanthropic behaviour to varying degrees, characteristics that make them potentially important as reservoirs of pathogens with human and animal health significance. In this work, three objectives have been defined to improve the understanding of white-toothed shrews as reservoirs of zoonotic pathogens and pathogens of currently unknown zoonotic potential.

Objective 1: Determining the presence of selected pathogens in white-toothed shrews from multiple countries in Europe and contribution to the description of the current distribution of white-toothed shrews in Germany.

Publication 1 & 3

Previous studies in Europe have mainly focused on the more abundant shrew species of the genus *Sorex*, particularly the common shrew (*Sorex araneus*), resulting in a lack of comprehensive pathogen studies on white-toothed shrews from Central Europe. The presence of *Leptospira* spp., *Coxiella burnetii*, *Brucella* spp. and arthropod-borne pathogens, such as *Neoehrlichia mikurensis*, *Bartonella* spp., *Babesia* spp., and *Anaplasma phagocytophilum*, were investigated in white-toothed shrews obtained from multiple European countries. The combination of molecular species identification based on the *cytochrome b* gene with metadata on the origin of the shrews provided important insights into the current distribution of the three white-toothed shrew species present in Germany.

Objective 2: Identification of the virome of white-toothed shrews using metagenomic high-throughput sequencing - revealing potential infections risks.

Publication 2, Review 1

Only a small fraction of the world's virosphere has been identified. This is especially valid for viral pathogens of shrews of the genus *Crocidura*. A metagenomic high-throughput sequencing approach was used to decipher the virome of white-toothed shrews from Central Europe.

Objective 3: Characterization of the Borna disease virus 1 (BoDV-1) reservoir by implementing the One Health approach in an area with the first detected human BoDV-1 cluster.

Publication 3, Review 1 & 2

The detection of the first regional cluster of lethal human BoDV-1 cases in a small village in Bavaria, Germany, facilitated the development and implementation of a One Health approach to investigate a range of factors associated with BoDV-1 infection. The objectives of this study were to improve the description of the clinical spectrum of human infections, investigate the local small mammal community to gain knowledge on the reservoir species in a defined area, and assess the role of the environment and ticks in transmission, to potentially identify sites of infection and to improve the understanding of BoDV-1 transmission.

IV. Results

Manuscripts, including their figures and tables are presented in the style of each respective journal. The same accounts for all references and abbreviations used, therefore they do not appear in the reference and abbreviation section of this thesis.

Publication 1: White-Toothed Shrews (Genus *Crocidura*): Potential Reservoirs for Zoonotic *Leptospira* spp. and Arthropod-Borne Pathogens?

White-Toothed Shrews (Genus Crocidura): Potential Reservoirs for Zoonotic Leptospira spp. and Arthropod-Borne Pathogens?

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Abstract: Three species of white-toothed shrews of the order Eulipotyphla are present in central Europe: the bicolored (*Crocidura leucodon*), greater (*Crocidura russula*) and lesser (*Crocidura suaveolens*) white-toothed shrews. Their precise distribution in Germany is ill-defined and little is known about them as reservoirs for zoonotic pathogens (*Leptospira* spp., *Coxiella burnetii*, *Brucella* spp., *Anaplasma phagocytophilum*, *Babesia* spp., *Neoehrlichia mikurensis* and *Bartonella* spp.). We investigated 372 *Crocidura* spp. from Germany (n = 341), Austria (n = 18), Luxembourg (n = 2) and Slovakia (n = 11). West European hedgehogs (*Erinaceus europaeus*) were added to compare the presence of pathogens in co-occurring insectivores. *Crocidura russula* were distributed mainly in western and *C. suaveolens* mainly in north-eastern Germany. *Crocidura leucodon* occurred in overlapping ranges with the other shrews. *Leptospira* spp. DNA was detected in 28/227 *C. russula* and 2/78 *C. leucodon* samples. Further characterization revealed that *Leptospira kirschneri* had a sequence type (ST) 100. *Neoehrlichia mikurensis* DNA was detected in spleen tissue from 2/213 *C. russula* samples. Hedgehogs carried DNA from *L. kirschneri* (ST 100), *L. interrogans* (ST 24), *A. phagocytophilum* and two *Bartonella* species. This study improves the knowledge of the current distribution of *Crocidura* shrews and identifies *C. russula* as carrier of *Leptospira kirschneri*. However, shrews seem to play little-to-no role in the circulation of the arthropod-borne pathogens investigated.

Keywords: shrew; reservoir; *Leptospira* spp.; *Anaplasma phagocytophilum*; *Neoehrlichia mikurensis*; *Babesia* spp.; *Bartonella* spp.; *Coxiella burnetii*; *Brucella* spp.; distribution

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1. Introduction

Shrews are small insectivorous mammals belonging to one of the largest mammalian families, the Soricidae [1]. Currently, 448 recent species are recognised, and new species continue to be discovered [2–4]. The family Soricidae is divided into three subfamilies: Soricinae (red-toothed shrews), Crocidurinae (white-toothed shrews) and Myosoricinae (African white-toothed shrews) [5,6]. Representatives of the subfamily Soricinae are most abundant in the Holarctic region, while crocidurine shrews evolved, and are only present, in Eurasia and Africa [7]. In central Europe, six species of red-toothed shrews (genus *Sorex*) and three species of white-toothed shrews (genus *Crocidura*) are described [1]. They differ not only by morphological traits such as tooth colour, but also in their behaviour and ecology. *Sorex* shrews prefer cool and moist, forest-covered habitats, while *Crocidura* shrews are found in dry and arid, more open spaces and can be commensal [8,9]. The most prevalent shrew species in Germany is the common shrew (*Sorex araneus*).

The exact distribution ranges of these shrews are scarcely described, especially for white-toothed shrews. The lesser white-toothed shrew (*Crocidura suaveolens* (Pallas, 1811)) and the bicolored white-toothed shrew (*Crocidura leucodon* (Hermann, 1780)) are sympatrically found mainly in southern and eastern Europe [10]. The current distribution range of the greater white-toothed shrew (*Crocidura russula* (Hermann, 1780)) expands from northern Africa through the Iberian Peninsula and France into Germany [11]. The colonization of Ireland [12] and Great Britain [13], as well as an ongoing northward [14] and eastward [15,16] expansion of *C. russula* within Germany, have been described. In areas newly colonised by *C. russula*, competition with the smaller *C. leucodon* and *C. suaveolens* has led to their local extinction [15–18].

The role of shrews as carriers for zoonotic pathogens is still understudied [19,20], and the few available studies focused mainly on the genus *Sorex* with the detection of several different hantaviruses of unknown zoonotic potential, such as the Seewis virus and the Asikkala virus [21,22]. Shrews of the Crocidurinae subfamily are even more poorly studied, except for *C. leucodon* as a proposed reservoir for Borna Disease Virus 1 (BoDV-1; species: *Orthobornavirus bornaense*; family: *Bornaviridae*) [23,24]. Other insectivorous species, such as the West European hedgehog (*Erinaceus europaeus*, Linnaeus, 1758), are well known major carriers of *Leptospira* spp. [25] and arthropod-borne pathogens [26–28]. Investigation in those species has provided good insight into potential pathogens carried by shrews, as they share habitats [1].

Leptospira spp. are obligate extracellular bacteria belonging to the phylum Spirochaetes. They are distributed worldwide and are associated with different reservoir host species, of which small mammals are the most important [29]. The bacteria are excreted into the environment via urine and may be transmitted via contaminated water and food or via direct contact to skin lesions or conjunctivae. Clinical manifestation of an infection with *Leptospira* spp. varies from mild flu-like symptoms to severe forms such as kidney organ failure (Morbus Weil) or encephalitis [29]. Studies of *Leptospira* spp. prevalence in small mammals in Germany have mainly focused on rodents and *Sorex* shrews [30], with the occasional detection of *Leptospira kirschneri* in *C. russula* and *C. leucodon* [31,32]. Interestingly, *Leptospira alstonii* was isolated from invasive *C. russula* in Ireland, with previous isolates only originating from non-mammal hosts from China, Japan and Malaysia [33]. Little is known about the presence or prevalence of *Leptospira* spp. in lesser and bicolored white-toothed shrews.

Anaplasma phagocytophilum, *Babesia* spp. and *Neoehrlichia mikurensis* are tick-borne pathogens transmitted by hard ticks, mostly of the genus *Ixodes* [34], causing febrile illness in humans, especially in immunocompromised patients [35]. High prevalence rates of tick-borne pathogens were described in the common shrew [36,37], but little is known about the prevalence of these pathogens in white-toothed shrews. *Bartonella* spp., most of which are considered zoonotic [35], are Gram-negative bacteria mainly transmitted by haemophilic arthropods (fleas, ticks and lice) and can persist in erythrocytes and endothelial cells in reservoir hosts (mainly rodents, cats (*Felis catus*) and game). The detection of *Bartonella* spp.

in shrews was described for *Sorex* spp. in Germany [37]. A newly described *Bartonella* strain, named *Bartonella florenciae*, was previously isolated from the spleen tissue of a *C. russula* from France [38,39].

The causative agent of "Q-fever", *Coxiella burnetii*, is a globally distributed Gram-negative bacterium that causes infertility and abortions, mainly in ruminants (cattle, goats and sheep), and is excreted in great numbers with birth materials and, to a lesser extent in milk, faeces and urine. Farmers, veterinarians and abattoir employees are high-risk groups for infection. Numbers on reported human infections have fluctuated between 55 and 416 cases per year in Germany since 2001 [40]. Ticks (in Germany, supposedly *Dermacentor marginatus*) can shed *C. burnetii* in their faeces and transmission could potentially occur through inhalation of faecal dust rather than by the tick bite [41]. There is only limited information about the role of small mammals in the infection cycle of *C. burnetii*. A seroprevalence of 19% was previously reported for rodents in the UK [42,43]. In the vicinity of Q-fever-positive farms, seroprevalences of up to 53% in wild rats have been observed [44]. Conversely, a study on small mammals from Slovakia reported a seroprevalence of only 2.2%, while investigated *Sorex* spp. had no antibodies against *C. burnetii* [45].

Brucella spp. are facultative intracellular bacteria that cause brucellosis, a severe disease in animals (reproductive failure and abortion) and humans (feverish multi-organ failure). Germany is considered to be free of bovine, ovine and caprine brucellosis. To maintain this status, its potential reintroduction by wildlife should be closely monitored. However, reported human cases are increasing [46]. Several years ago, a new *Brucella* species, *Brucella microti*, was isolated from common voles in central Europe [47] and has since been detected in other wildlife [48,49]. Previous studies identified that 8% of all investigated soricine shrews [50] were *Brucella* spp.-positive, but so far no data are available on the presence of this pathogen in *Crocidura* spp. from Germany.

As data on the current distribution of greater, lesser and bicolored white-toothed shrews in Germany are incomprehensive and knowledge on their role as carriers for pathogens with zoonotic potential is limited, the objectives for this study were to (i) contribute to the current knowledge on the distribution of white-toothed shrews in Germany, (ii) detect and characterise *Leptospira* spp. in white-toothed shrews and (iii) evaluate white-toothed shrews as reservoirs for arthropod-borne pathogens and compare the findings to European hedgehogs.

2. Materials and Methods

2.1. Collection and Dissection of Shrews and Hedgehogs

Shrews from Germany, Luxembourg, Austria and Slovakia were collected between 1999 and 2021 (Figure 1, Table S1). The majority of these originated from a citizen-science-based project, where the public was asked to send in shrews trapped by cats or found dead. Additionally, shrews were trapped as by-catch during various rodent monitoring studies and pest control measures in Germany [32,51]. European hedgehogs were collected at a rescue center in Offenbach, Germany. Information on collection date and site were recorded; the latter was defined by common postal code as it was the most precise information available for specimens from prey of cats. All animals were transported on dry ice to the laboratory and stored at −20 °C until further processing. Kidney and spleen tissues were taken during a standardised necropsy procedure [52] and stored at −20 °C. Morphological metadata on body weight and sex were taken during necropsy (Table S2).

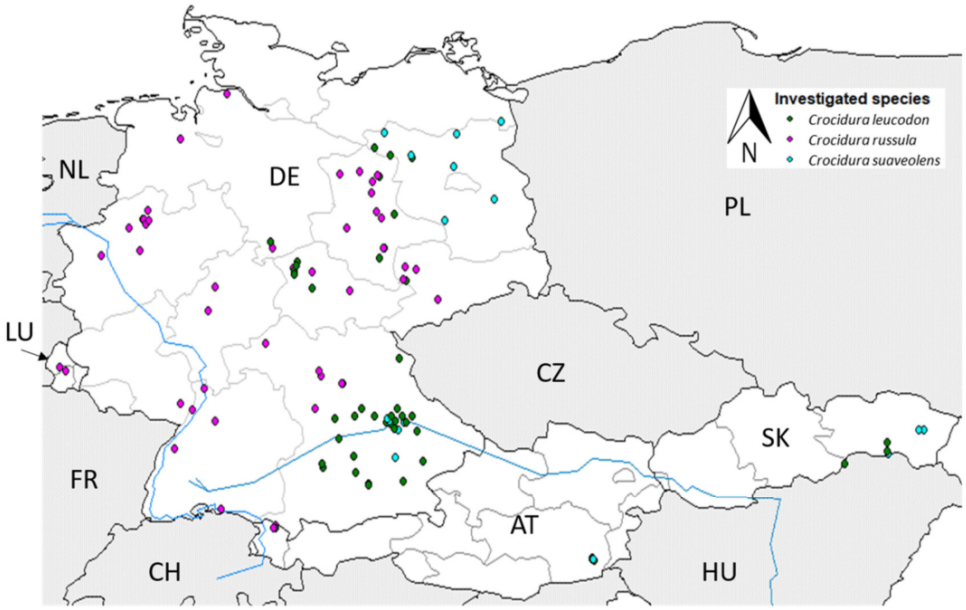

Figure 1. Origin of the investigated white-toothed shrews from Germany (n = 341), Luxembourg (n = 2), Austria (n = 18) and Slovakia (n = 11) based on common postal code; per trapping site, each detected species is represented by one dot. NL: the Netherlands; LU: Luxembourg; FR: France; DE: Germany; CH: Switzerland; AT: Austria; CZ: Czech Republic; PL: Poland; SK: Slovakia; HU: Hungary.

2.2. Nucleic Acid Extraction

Nucleic acids were extracted from kidney and spleen tissue using a Nucleo Mag Vet Kit (Macherey & Nagel, Düren, Germany) and a KingFisher™ Flex Purification System (Thermo Fisher Scientific, Darmstadt, Germany) according to the manufacturer's instructions.

2.3. Molecular Species Identification

Species identification for each shrew was performed based on the molecular analysis of the almost-complete *cytochrome b* gene and sequence comparison to GenBank entries as previously described [53].

2.4. Polymerase-Chain-Reaction-Based Screening for Leptospira spp. DNA

Kidney-derived DNA was screened in pools of two for the presence of *Leptospira* spp. DNA with a real-time PCR (qPCR) targeting the *lipl*32 gene (expected amplicon size: 242 base pairs, bp), encoding for an outer membrane lipoprotein [54]. Positive pools were retested for each individual, and samples with a cycle threshold (Ct) value below 41 were considered as *Leptospira*-positive. As positive control, DNA of a laboratory strain of *L. kirschneri* serovar Grippotyphosa was used [55]. Three *C. leucodon* samples were investigated previously by conventional *lipl32* gene PCR [32].

2.5. Multilocus Sequence Typing of Leptospira spp.

Multilocus sequence typing (MLST) of seven target genes, *glmU* (amplicon size: 650 bp), *pntA* (621 bp), *sucA* (640 bp), *tpiA* (639 bp), *pfkB* (588bp), *mreA* (791 bp) and *caiB* (650 bp), was performed for samples with a Ct value < 36 following the scheme from Boonsilp et al. [56] considering modifications as described [54].

2.6. Amplification and Sequencing of the secY Gene of Leptospira spp.

For samples with a Ct value > 36 or with incomplete MLST results, a conventional PCR targeting the *secY* gene (657 bp) was performed to determine *Leptospira* species as

previously described [54]. As positive control, DNA of a laboratory strain of *L. interrogans* serovar Icterohaemorrhagiae was used [55].

PCR products were prepared with DNA Gel Loading Dye (6x) (Thermo Fisher Scientific, Darmstadt, Germany) for gel electrophoresis in 2% agarose, and gels were stained with HDGreen Plus DNA Stain (Intas Science Imaging Instruments GmbH, Göttingen, Germany). Amplification products were visualised by UV light using the UVP GelSolo streamlined gel documentation (Analytik Jena AG, Jena, Germany). The samples were purified for sequencing using a NucleoSpin Gel and PCR clean-up kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) as recommended by the manufacturer. The sequences were trimmed using Bionumerics v.7.6.1. (Applied Maths Inc., Austin, TX, USA) and compared to available data in GenBank with the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 7 August 2022). The obtained sequences were uploaded to GenBank (accession numbers: OQ865429–OQ865435).

2.7. Polymerase-Chain-Reaction-Based Screening for Arthropod-Borne Pathogens, Coxiella burnetii and Brucella spp.

The presence of *Bartonella* spp. was evaluated in individual spleen DNA samples by conventional PCR targeting the nicotinamide adenine dinucleotide hydrogen dehydrogenase (NADH) subunit (nuoG) with an amplicon size of 346 bp [57]. DNA from a cultured *B. henselae* Marseille strain was used as positive control. Positive samples were further analysed by PCR targeting the *gltA* gene (amplicon size: 378 bp) [57,58]. Positive samples were purified and sequenced commercially (Interdisziplinäres Zentrum für Klinische Forschung, Leipzig, Germany). The obtained sequences were uploaded to GenBank (accession numbers: OQ865426–OQ865428). Spleen-derived DNA pools of two individuals were screened with qPCRs for the presence of *Anaplasma phagocytophilum* DNA targeting the *msp2* (major surface protein 2) gene (amplicon size: 77 bp) [59] and *Neoehrlichia mikurensis* DNA targeting the *groEL* gene (amplicon size: 99 bp) as previously described [60]. As positive controls, we used DNA from an *A. phagocytophilum* culture and DNA from a *N. mikurensis* positive yellow-necked field mouse (*Apodemus flavicollis*) from Leipzig, Germany, that was trapped in 2016 [61], respectively. Positive pools were retested on an individual level. Spleen DNA samples in pools of three were used for the detection of *Babesia* spp. DNA by conventional PCR targeting a fragment (411–452 bp) of the *18S rRNA* gene [62]. For the detection of *Coxiella burnetii* DNA and *Brucella* spp. DNA, all individual spleen-derived DNA samples were screened using a qPCR targeting the multicopy insertion element IS1111 [63] or the *bcsp31* gene [64], respectively.

2.8. Statistical Analysis

All statistics were performed in the GraphPad Prism Software v. 4.0 (GraphPad Software Inc., San Diego, CA, USA). Mean prevalence and confidence intervals (95% CI) for *Leptospira* spp. were determined using the Clopper and Pearson method with an alpha value of 0.05. For the prevalence of *Leptospira* and the sex of different *Crocidura* species, Fisher's exact test was used to test independence. Tests were considered to be significant if p (probability) < 0.05.

2.9. Generation of Maps

Maps were generated using Karten-Explorer v. 2.21 (Friedrich-Loeffler-Institut (FLI), Bundesforschungsinstitut für Tiergesundheit Copyright © 2022, Greifswald, Insel Riems, Germany). The German federal states were grouped into four regions: southwest, northwest, northeast and southeast, for the evaluation of the geographical distribution of white-toothed shrews (Figure 2).

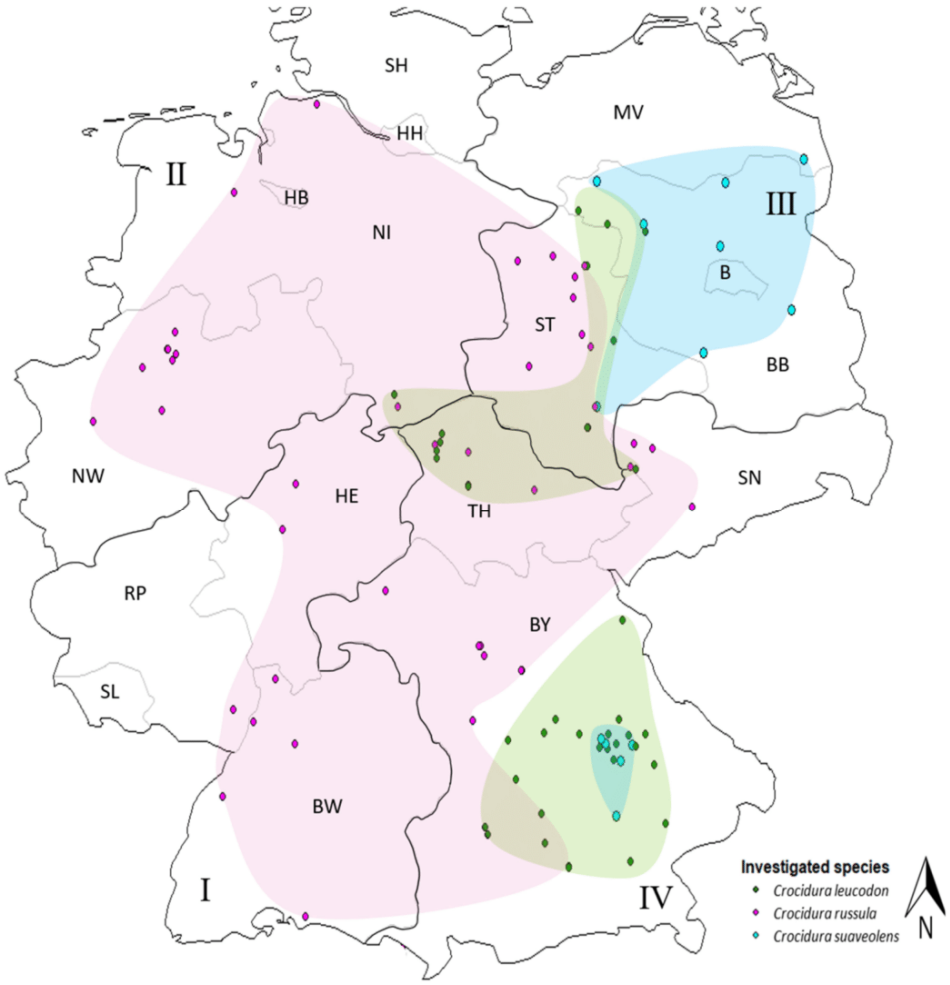

Figure 2. Distribution of investigated white-toothed shrews from Germany: greater white-toothed shrew (*Crocidura russula*, purple), bicolored white-toothed shrew (*Crocidura leucodon*, green), lesser white-toothed shrew (*Crocidura suaveolens*, blue); per trapping site, each detected species is represented by one dot. I Southwest: SL: Saarland, RP: Rhineland–Palatinate, BW: Baden–Wuerttemberg, HE: Hesse; II Northwest: NW: North Rhine–Westphalia, NI: Lower Saxony, HB: Bremen, HH: Hamburg; III Northeast: ST: Saxony–Anhalt, BB: Brandenburg, B: Berlin; MV: Mecklenburg–Western Pomerania; IV Southeast: BY: Bavaria, TH: Thuringia, SN: Saxony.

3. Results

3.1. Distribution of White-Toothed Shrews

In total, 341 shrews were collected between 2002 and 2021 in Germany: 235 greater white-toothed shrews (68.9%; 99 males, 122 females, 14 sex not determined (s.n.d.), 83 bicolored white-toothed shrews (24.3%; 38 males, 42 females, three s.n.d.) and 23 lesser white-toothed shrews (6.7%; 12 males, 11 females) (Figure 1).

The shrews originated from the southwest (n = 9), northwest (n = 103), northeast (n = 110) and southeast (n = 118) of Germany (Figure 2). *Crocidura russula* was the most abundant species, especially in the western parts of Germany—northwest: 99% (n = 103) and southwest: 100% (n = 9). Only one *C. leucodon* (1%) was collected in the southeast of Lower Saxony close to the Harz mountain range (Table S1). In the eastern half of Germany, the situation was more diverse. All three species could be found in the northeast, with 70% *C. russula* (n = 77), 13.6% *C. leucodon* (n = 15) and 16.4% *C. suaveolens* (n = 18). *Crocidura russula* was still the predominant species in northeast Germany, but it was not

collected in the state of Brandenburg (BB), which is far northeast, where mainly *C. suaveolens* was found (78.3% of all investigated *C. suaveolens*). In the southeast, especially in the south of Bavaria, *C. leucodon* was the most prominent (56.8%, n = 67), and *C. russula* (39%, n = 46) was mainly found in Franconia and further north. Of all the collected white-toothed shrews from the southeast 4.2% were *C. suaveolens* (n = 5) (Figure 2, Table S1). The species composition varied per site. The occurrence of *C. russula* and *C. leucodon* overlapped at five sites (Figure 2), and *C. leucodon* and *C. suaveolens* overlapped at four sites. *Crocidura russula* and *C. suaveolens* were only found together at one site in the northeast of Germany. We did not find all three species at the same site. A few white-toothed shrews from neighbouring countries in central Europe were included in our study: two *C. russula* from Luxembourg, two *C. russula* and one *C. leucodon* from Vorarlberg, Austria, three *C. leucodon* and twelve *C. suaveolens* from the eastern state of Steiermark, Austria, and five *C. leucodon* and six *C. suaveolens* from Slovakia (Figure 1).

3.2. Detection and Sequence Type Identification of *Leptospira* spp.

Leptospira spp. DNA was detected in kidney samples from 28 out of 227 *C. russula* (12.3%, 95% CI: 8.6–17.3) and three out of 81 *C. leucodon* (3.7%, 95% CI: 0.8–10.7) samples from Germany (Table 1).

Table 1. Results for the detection of *Leptospira* spp. with *lipl*32-qPCR in kidney tissue and *Neoehrlichia mikurensis* (*groEL*-qPCR), *Anaplasma phagocytophilum* (*msp*2-qPCR) and *Coxiella burnetii* (multicopy IS1111 element-qPCR), *Brucella* spp. (*bcsp31*-qPCR) and conventional PCR results for the detection of *Babesia* spp. (*18S* rRNA) and *Bartonella* spp. (*nuoG*-PCR) in spleen tissue of white-toothed shrews from Germany collected between 2002–2021.

Species	Number of *Leptospira* DNA-Positive/Total Number of Tested Individuals (Percentage, 95% CI *)	Number of *N. mikurensis* DNA-Positive/Total Number of Tested Individuals (Percentage, 95% CI *)	Number of *A. phagocytophilum*, *C. burnetii*, *Brucella* spp., *Babesia* spp. and *Bartonella* spp. DNA-Positive/Total Number of Tested Individuals (Percentage, 95% CI *)
Greater white-toothed shrew *** *(Crocidura russula)*	28/227 (12.3%, 8.6–17.3)	2/213 (0.9%, 0–3.6)	0/213 (0%, 0–2.1)
bicolored white-toothed shrew *** *(Crocidura leucodon)*	3/81 ** (3.7%, 0.8–10.7)	0/80 (0%, 0–5.5)	0/80 (0%, 0–5.5)
Lesser white-toothed shrew *** *(Crocidura suaveolens)*	0/22 (0%, 0–17.6)	0/21 (0%, 0–18.2)	0/21 (0%, 0–18.2)

* CI: confidence interval. ** including three *C. leucodon* previously investigated by Jeske et al. [32] *** *C. leucodon*, *C. russula* and *C. suaveolens* from Luxembourg, Austria and Slovakia tested negative for all investigated pathogens.

All of the *C. russula* and *C. leucodon* samples from Luxembourg and Austria and all of the 22 *C. suaveolens* tested negative for the presence of *Leptospira* spp. DNA (0%, 95% CI: 0–17.6). Thus, the prevalence was significantly lower in *C. leucodon* and *C. suaveolens* compared to *C. russula* ($p = 0.003$). Out of the 28 *lipl*32 qPCR-positive *C. russula*, six were identified as *Leptospira kirschneri* by sequencing the *secY* PCR product. MLST was successful for an additional six individuals (*C. russula*) and were determined to be the same sequence type: *Leptospira kirschneri* ST 100. The sequencing of the *sec*Y PCR product of the *lipl*32 qPCR-positive *C. leucodon* was not possible, which was most likely due to the poor sample DNA quality. There was no significant difference in the prevalence between female (10.3%, 95% CI: 5.8–17.2) and male *C. russula* (14.6%, 95% CI: 8.8–23.1) ($p = 0.337$).

Leptospira spp. DNA-positive individuals originated from 15 trapping sites from across Germany (Figure 3). The prevalence of *Leptospira kirschneri* at the different sites varied between 5.6% and 40% (mean $\bar{x} = 25\%$); sites with less than four individuals were excluded (mean $\bar{x} = 13$; 4–33 individuals per site). The hedgehog investigation revealed that four

of the 42 (9.5%, 95% CI: 3.2–22.6) animals were *lipl*32 qPCR-positive, which were further characterised as *L. kirschneri* (ST 100) and *L. interrogans* (ST 24).

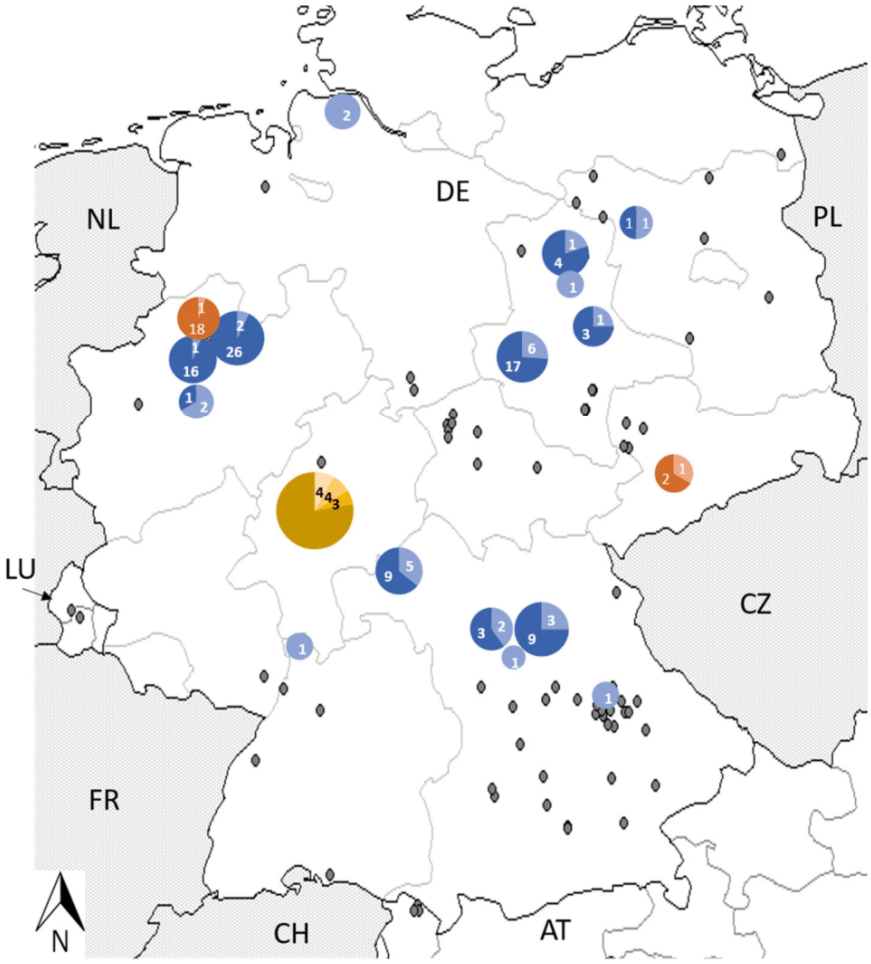

Figure 3. Detection of *Leptospira kirschneri* DNA (blue) and *Neoehrlichia mikurensis* DNA (orange) in white-toothed shrews. Numbers of positive individuals are indicated by a brighter colour. Trapping sites with no detection of any investigated pathogens are marked in grey. Investigations into hedgehogs are shown in yellow (four *Leptospira* spp. DNA, four *A. phagocytophilum* DNA and three *Bartonella* spp. DNA positive hedgehogs, with no co-infection).

3.3. PCR Analysis for Arthropod-Borne Pathogens, Coxiella burnetii and Brucella spp.

The PCR screening of spleen samples of 213 *C. russula*, 80 *C. leucodon* and 21 *C. suaveolens* from Germany detected *Neoehrlichia mikurensis* DNA in two female *C. russula* (0.9%, 95% CI: 0–3.6) samples, one from southeast Germany and the other one from northwest Germany (Figure 3). None of the 80 investigated *C. leucodon* (0%, 95% CI: 0–5.5) and 21 *C. suaveolens* (0%, 95% CI: 0–18.2) tested positive for *N. mikurensis* DNA. None of the investigated shrews were positive for *Babesia* spp., *A. phagocytophilum*, *Bartonella* spp., *Brucella* spp. or *C. burnetii* DNA (Table 1). The shrews from Austria and Slovakia were negative for all pathogens. The shrews from Luxembourg were not investigated due to a lack of spleen tissue. The hedgehog group indicated the presence of *A. phagocytophilum* in four of the 42 (9.5%, 95% CI: 3.2–23.6) animals. Three of the 42 (7.1%, 95% CI: 1.8–20.0) hedgehogs tested positive for *Bartonella* spp., two being typed as *B. clarridgeiae* strain 73 and one as uncultured *Bartonella* spp. None of the 42 hedgehogs tested positive for *N. mikurensis*, *Babesia* spp., *Brucella* spp. and *C. burnetii* DNA.

4. Discussion

4.1. Current Distribution of White-Toothed Shrews in Germany

The collection of 341 white-toothed shrews allowed, albeit with limitations due to the heterogenous sampling, an update on the current distribution of *Crocidura* spp. in Germany. The latest comprehensive survey on the distribution of white-toothed shrews in Germany covered only southeast Germany (Bavaria) [65] and was mainly based on the identification of skeletal remains in owl pellets. With our citizen science project, which exploited cats' aversion to consume shrews, we were able to collect fresh carcasses to accurately identify the species using molecular techniques and to perform an initial screening of their accompanying pathogens, which allowed us to determine health risks to cats and their owners.

Over the past decades, multiple studies [9,66–69] have monitored the distribution boundaries of white-toothed shrews on local levels [14,70–72], describing fluctuations in total white-toothed shrew numbers [17] and uncertain boundaries. The core distribution range of *C. russula* expands from the western European countries into central Germany and is slowly expanding further east [15,73,74]. The collection of *C. russula* in our study in western and southeastern Germany coincided with the easternmost expansion into Franconia, Bavaria [65]. In regions where *C. russula* occurred, *C. russula* predominated over the other two species, which may have led to the local extinction of *C. suaveolens* as they are considered parapatric species [15,18,74]. Whether this is solely due to the size difference between the larger *C. russula* and the smaller *C. suaveolens* or due to differences in adaptations to synanthropic habitats and climate conditions, as *C. russula* copes better with drier, hotter summers, and therefore, out-competition is still under debate [15,18]. The same applies to *C. leucodon*, as *C. russula* was primarily found in former typical *C. leucodon* habitats [18,74–76]. The eastwards expansion of *C. russula* and the replacement of *C. leucodon* has also been observed in Switzerland [16] and Austria [8,77]. Although limited by number, we observed the same trend with *C. russula*, it being found in the northwest of Austria, while in the east of Austria so far only *C. leucodon* and *C. suaveolens* were collected. We primarily detected *C. suaveolens* in the northeastern part of Germany, supporting the westwards expansion trend described by Jentzsch and Trost [78]. *Crocidura suaveolens* were sporadically found in the southeast, but not at all in the western parts of Germany. Similarly, the absence of *C. leucodon* from the southwest was consistent with previous reports describing a decline in *C. leucodon* occurrence in the western half of Germany [68,75,76]. Information on the exact origin of an individual is needed to determine territory size and sym- and parapatry, which was not possible with our sample collection as it was greatly influenced by the cats' behaviour. We decided to use postal codes as the smallest common spatial factor. All three species were not found together, but the co-occurrence of *C. leucodon* and *C. russula* versus *C. leucodon* and *C. suaveolens* was almost equally frequent (n = 5 vs. n = 4); however, *C. suaveolens* and *C. russula* were only collected together at one site in northeastern Germany. Between 1995 and 2010, the co-occurrence of all three species was described for east Thuringia [74] and west Saxony [18]. There are multiple possible explanations for the ongoing fluctuation and expansion of the species' distribution ranges, including ongoing postglacial expansion [5], man-made factors due to alterations in land use and climate [79] or simply the translocation of individuals [16]. Anthropogenic movement has a great influence in the range expansion, as shrews might be transported via feed (e.g., haystacks) or soil. Once translocated, shrews easily establish new colonies [80–82], as seen in the introduction of the greater white-toothed shrew to Ireland, most likely due to human activity, in the early 21st century [12]. Since then, *C. russula* has expanded at a pace of 15 km/year, which is much faster than described for continental Europe.

4.2. Detection and Characterization of Leptospira spp. in White-Toothed Shrews

In regard to small mammals, previous studies of *Leptospira* prevalence were mainly focused on rodents and soricine shrews. Depending on the shrew species and geographic region, previous studies describe a mean *Leptospira* prevalence of 3.0% (range 0–3.4%; crowned shrew, *Sorex coronatus*), 6.8% (range 0–21.1%; pygmy shrew, *Sorex minutus*) and 15.5% (range 0–23.5%; common shrew, *Sorex araneus*) [30].

The current knowledge on *Leptospira* in crocidurine shrews in central Europe is scarce. *Leptospira* spp. was detected in *C. russula* already in the 1970s [83]. In Germany, *Leptospira kirschneri* was found in *Crocidura russula* [84] and *Crocidura leucodon* [32], but no further sequence typing was performed. Here, we detected *Leptospira kirschneri* in 28 *C. russula* and two *C. leucodon* with a mean prevalence of 25% (5.6–40%) at 15 trapping sites. *Leptospira* spp. was irregularly distributed in Germany, as demonstrated by its absence in white-toothed shrews from Saxony (this study, [85]). The irregular distribution and broad variation in the prevalence per trapping site might be caused by a biased sample size per site and the geographic origin of the samples. Water and moist areas play an important role in the maintenance and spread of *Leptospira* spp. outside their animal hosts [29]; crocidurine shrews prefer more open, arid habitats, which might explain the lower *Leptospira* spp. prevalence compared to *Sorex* spp. and rodents. The observed difference in prevalence between *C. russula* and *C. leucodon* could be due to the differences in habitat use between the species. *Crocidura russula* is a range-expanding invader [86] and may therefore have a higher exposure to *Leptospira*. Unfortunately, a comparison of the exact habitat use between the shrew species was not possible due to our sampling method. Although *Leptospira kirschneri* has been described as the most abundant genomospecies in small mammals, Jeske et al. [32] detected *Leptospira borgpetersenii* in sympatric rodents from trapping sites, where *L. kirschneri* was found in *C. leucodon*. Interestingly, the investigated hedgehogs carried two *Leptospira* species, *L. kirschneri* ST 100 and *Leptospira interrogans* ST 24, with the latter one commonly found in forest-dwelling rodents such as yellow-necked field mice and wood mice (*Apodemus sylvaticus*) [30].

MLST allowed us to determine the ST of *Leptospira* spp., and it is widely used to evaluate the spread of a specific pathogen within a population to distinguish detection in maintenance hosts from spill-over and host-switch events. In small mammal populations, different sequence types are seen within the same species and the same ST in different animal species. Common shrews from various locations in Germany have been shown to carry *Leptospira kirschneri* of two different sequence types (ST 110, ST 136) as well as *Leptospira borgpetersenii* of ST 146 [30]. *Leptospira kirschneri* ST 110 is strongly associated with voles of the genus *Microtus* and is the most common source of leptospirosis outbreaks in strawberry pickers in Germany [30]. Interestingly, we found only a single *Leptospira kirschneri* ST (ST 100) in all the *C. russula* samples from the different trapping sites across Germany, suggesting a possible host species specificity and may identify *C. russula* as maintenance host rather than spill-over host. However, this ST was also found in a European hedgehog (this study) and was previously isolated from a Portuguese house mouse (*Mus musculus*) [87]. This ST has been associated to the serovar Mozdok, a serovar that is widely distributed in small mammals (mainly *Apodemus agrarius*) in central Europe [88], which causes canine leptospirosis [89] and is also associated with human infections [90]. Further investigations on sympatric small mammals from the same trapping sites are needed to determine how widespread ST 100 is within the small mammal community. Unfortunately, for the publicly available ST 100 isolate (*Leptospira* isolate 15-LE00367-0 [91]) from Germany, the host species and its precise origin in Lower Saxony, Germany, is not specified.

4.3. Identification of White-Toothed Shrews as Reservoirs for Arthropod-Borne Pathogens

A high prevalence of tick-borne pathogens has been described for common shrews [36,37], but little is known about the prevalence of these pathogens in white-toothed shrews. A comparable study from Spain found *A. phagocytophilum* in one of six *C. russula* samples [92], whereas a previous study from Germany did not detect *A. phagocytophilum*, *Babesia* spp. and

N. mikurensis in any *C. russula* sample [60]. Even though our sample size (n = 372) was much larger than that of previous studies (n = 4), we still did not detect *A. phagocytophilum* in any white-toothed shrew. *Anaplasma phagocytophilum* is present in the small mammal community in Germany, as confirmed here by the prevalence of about 10% in European hedgehogs (this study, [27]) and in crowned shrews and bank voles (*Clethrionomys glareolus*) [37]. We detected *N. mikurensis* DNA in two *C. russula* samples at different urban sites in northwestern and southeastern Germany, a finding that seems to be in contradiction to the assumption of previous studies that insectivores do not play a role in the transmission and maintenance of *N. mikurensis* [93]. The detection and further characterization of *Bartonella* spp. from soricine shrews in Germany revealed host-specific *Bartonella taylorii*-associated strains [37,94]. So far, *Bartonella* spp. have only been detected in *Crocidura* spp. outside of Germany [95,96], e.g., the detection of the new species *Bartonella refiksaydamii* in the blood of a lesser white-toothed shrew from northwestern Turkey by Celebi et al. [97]. In this study, we did not detect *Bartonella* spp. DNA in any of the white-toothed shrews, but we identified the *Bartonella clarridgeiae* strain 73 and an "uncultured *Bartonella* spp." in the hedgehogs. *Bartonella clarridgeiae* is commonly present in cats [38], is transmitted by cat fleas (*Ctenocephalides felis*) and was once found in an asymptomatic blood donor in Brazil [98]. The role of small mammals and shrews in particular for the transmission of *Babesia* spp. and *Coxiella burnetii* is ill-defined. In our study, we did not detect *Babesia* spp. DNA in any of the crocidurine shrews or hedgehogs, even though Bown et al. [36] reported a *Babesia microti* prevalence of 30.3% in common shrews occupying the same habitat as field voles (30.4% *B. microti*-prevalence). Despite reports of a high seroprevalence for *C. burnetii* in rodents [42], all of the insectivores tested here were negative according to the PCR analysis. Assuming that small mammals are exposed to *C. burnetii*, shrews and hedgehogs do not seem to play a role as reservoirs. Fleas collected from *C. suaveolens* were tested for the presence of *C. burnetii* and rickettsiae, but they did not contain any respective DNA [99]. Previous detection of *Brucella* spp. in soricine shrews [50] could not be demonstrated for crocidurine shrews, as all of the insectivores tested here were negative.

Little is known about ectoparasites on shrews, but a white-toothed-shrew specific "ectoparasite milieu" [99,100], reducing the possible transmission of arthropod-borne pathogens from other (small mammal) species, might be an explanation for the observed low pathogen prevalence. Even though different life stages of *Ixodes ricinus* and *Dermacentor reticulatus* could be collected from *C. leucodon* and *C. suaveolens* trapped in Slovakia, the numbers were much lower than those from sympatric rodent species [101].

5. Conclusions

This study provides an update on the current distribution of white-toothed shrews in Germany. Altogether, white-toothed shrews seem to play a minor role in the transmission of *Leptospira* spp. and arthropod-borne pathogens. However, our study was limited by its sample size and sampling approach, heavily relying on the cooperation of the public. In the future, a more systematic and longitudinal study, ideally in a One Health setting, is needed to evaluate the potential infection risks of shrews and hedgehogs. The short life expectancy and high turnover rate of local shrew populations, including frequent extinction and fast recolonization events as described for *C. russula* [82], potentially influencing pathogen persistence in shrew communities, should be taken into account.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens12060781/s1, Table S1: Information on the origin of investigated shrews.; Table S2: Metadata on investigated white-toothed shrews and hedgehogs.

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Institutional Review Board Statement: Small mammals were trapped with snap traps by forest institutions within their professional duties and during ecological investigations (permit number: TH (22-2684-04-15-105/16). Ethical review and approvals were obtained for live animal trappings (permit numbers: ST: 42502-2-1548 UniLPZ; NW:84-02.04.2015.A279). The majority of the small mammals originated from a citizen-science-based project (cat preys, found dead); therefore, no further permits were required.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are presented within the manuscript and its Supplementary Materials. Sequence data were uploaded to GenBank (accession numbers: OQ865426-OQ865435).

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Publication 2: Detection of novel orthoparamyxoviruses, orthonairoviruses and an orthohepevirus in European white-toothed shrews

Detection of novel orthoparamyxoviruses, orthonairoviruses and an orthohepevirus in European white-toothed shrews

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Detection of novel orthoparamyxoviruses, orthonairoviruses and an orthohepevirus in European white-toothed shrews

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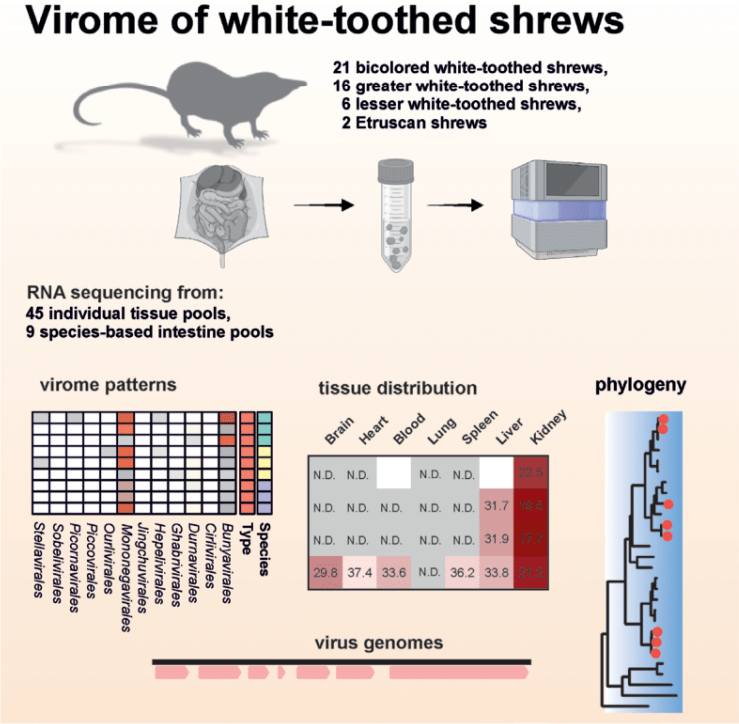
Graphical Abstract



Abstract

While the viromes and immune systems of bats and rodents have been extensively studied, comprehensive data are lacking for insectivores (order Eulipotyphla) despite their wide geographic distribution. Anthropogenic land use and outdoor recreational activities, as well as changes in the range of shrews, may lead to an expansion of the human–shrew interface with the risk of spillover infections, as reported for Borna disease virus 1. We investigated the virome of 45 individuals of 4 white-toothed shrew species present in Europe, using metagenomic RNA sequencing of tissue and intestine pools. Moderate to high abundances of sequences related to the families *Paramyxoviridae*, *Nairoviridae*, *Hepeviridae* and *Bornaviridae* were detected. Whole genomes were determined for novel orthoparamyxoviruses (*n*=3), orthonairoviruses (*n*=2) and an orthohepevirus. The novel paramyxovirus, tentatively named Hasua virus, was phylogenetically related to the zoonotic Langya virus and Mòjiāng virus. The novel orthonairoviruses, along with the potentially zoonotic Erve virus, fall within the shrew-borne Thiafora virus genogroup. The highest viral RNA loads of orthoparamyxoviruses were detected in the kidneys, in well-perfused organs for orthonairoviruses and in the liver and intestine for orthohepevirus, indicating potential transmission routes. Notably, several shrews were found to be coinfected with viruses from different families. Our study highlights the virus diversity present in shrews, not only in biodiversity-rich regions but also in areas influenced by human activity. This study warrants further research to

characterize and assess the clinical implications and risk of these viruses and the importance of shrews as reservoirs in European ecosystems.

Impact Statement

The detection of the zoonotic Langya virus in white-toothed shrews in China, as well as studies on the zoonotic Borna disease virus 1 in bicolored white-toothed shrews in Germany, have stimulated interest in white-toothed shrews as reservoirs for pathogens. Here, we used metagenomic sequencing to reveal the virome of white-toothed shrews in Europe. This has resulted in the description and phylogenetic classification of several novel viruses of the families *Paramyxoviridae*, *Nairoviridae* and *Hepeviridae*. We could demonstrate a high diversity of viruses and co-infections in synanthropic white-toothed shrews. Public awareness of pathogens in shrews is important for establishing targeted countermeasures for risk reduction while maintaining biodiversity.

DATA SUMMARY

Viral genomes and raw read data were uploaded to GenBank using the accessions OR713845–OR713892 (BioProject: PRJNA1028379).

INTRODUCTION

Knowledge of pathogen diversity in wildlife species is essential to be prepared for the next pandemic, a key task of modern virology [1]. Current estimates suggest that 75% of emerging human pathogens originate from (wild) animals [2, 3]. Small mammals, especially rodents and bats, are well-known reservoirs of zoonotic viruses [4–6], but little is known about the virosphere of insectivore species, especially shrews [7]. Shrews (Mammalia: Eulipotyphla: Soricidae) are species-rich and phylogenetically ancient (>45 million years) [8]. Three subfamilies are defined within the family Soricidae: Soricinae (red-toothed shrews), Crocidurinae (white-toothed shrews) and Myosoricinae (African white-toothed shrews). At least 242 species from 10 genera with an almost global distribution belong to the Crocidurinae subfamily, and the great diversity is increasing with the discovery of new species (Fig. 1) [9].

Primarily, four synanthropic species of white-toothed shrews are found in Europe: the bicolored white-toothed shrew (*Crocidura leucodon*), the greater white-toothed shrew (*Crocidura russula*), the lesser white-toothed shrew (*Crocidura suaveolens*) and the Etruscan shrew (*Suncus etruscus*) [8]. *C. russula* originates from North Africa and is currently distributed across Western Europe towards Fennoscandia and the Czech Republic [10–12]. *C. leucodon* is found from northern France through southern Europe to the Caspian Sea. The Etruscan shrew, one of the smallest recent living mammals with a body weight <2 g, is found mainly in southern Europe with a scattered distribution across parts of Africa and Asia (Fig. 1) [8]. The phylogenetic relationships among shrew species remain incompletely understood, with several species complexes, including the *C. suaveolens* sf. species complex, which shows a wide but fragmented distribution from the Atlantic coast to China [8].

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Keywords: *Crocidura*; insectivores; metagenome; reservoir; virome; white-toothed shrews.
Abbreviations: BoDV-1, Borna disease virus 1; CCHFV, Crimean-Congo haemorrhagic fever virus; CENV, Cencurut virus; DewV, Denwin virus; ERVEV, Erve virus; GamV, Gamak virus; HasV, Hasua virus; HeV, Hendra virus; ICTV, International Committee on Taxonomy of Viruses; LayV, Langya virus; LechV, Lechcodon virus; MeliV, Melian virus; MojV, Mòjiāng virus; NiV, Nipah virus; ORF, open reading frame; RASV, Rasenna virus; REGV, Regana virus; ResV, Resua virus; RT-qPCR, quantitative reverse transcription PCR; TFAV, Thiafora virus.
Viral genomes and raw read data were uploaded to GenBank using the accessions OR713845–OR713892 (BioProject: PRJNA1028379).
†These authors contributed equally to this work
Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Seven supplementary figures and three supplementary tables are available with the online version of this article.

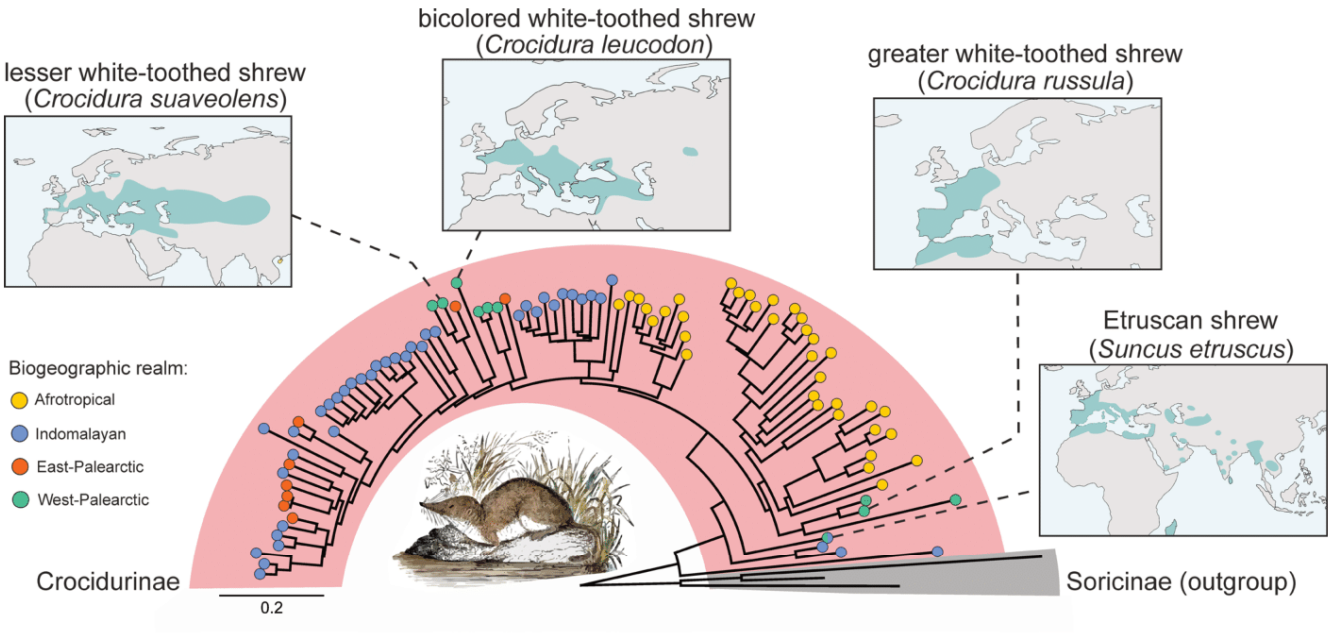

Fig. 1. Phylogenetic relationships and biogeographic distribution of extant white-toothed shrews. The phylogenetic tree is based on all available *cytochrome b* sequences from white-toothed shrews (subfamily Crocidurinae) and a selected outgroup of red-toothed shrews (subfamily Soricinae) (IQ-TREE2; version 2.2.2.6). The biogeographic distribution of these animals can be broadly grouped into four realms: Afrotropical, Indomalayan, East-Palearctic and West-Palearctic. The geographical range of the four Crocidurinae species that can be found in Europe is highlighted in the maps, according to [8]. Note the phylogenetic distances between *Suncus etruscus*, *Crocidura russula* and *Crocidura leucodon*/*Crocidura suaveolens*.

At present, knowledge of pathogens in European shrews, especially white-toothed shrews, is limited, apart from intensive studies of *C. leucodon*, the natural reservoir for zoonotic Borna disease virus 1 (BoDV-1) [13], which causes fatal encephalitis in both humans and domestic animals [14, 15]. However, sporadic detection of orthonairoviruses and paramyxoviruses has been reported.

Since the first report of Thiafora virus (TFAV) isolated from a *Crocidura* sp. shrew in Senegal in 1971, the number of new orthonairoviruses detected in shrews increased [16]. Erve virus (ERVEV) was identified in *C. russula* from France [16–18]. More recently, Lamusara virus and Lamgora virus have been described in the Goliath shrew (*Crocidura goliath*) from Gabon [19] and Cencurut virus (CENV) in the Asian house shrew (*Suncus murinus*) from Singapore [20]. All these viruses belong to the Thiafora virus genogroup, which is distantly related to the zoonotic Crimean-Congo haemorrhagic fever virus (CCHFV). CCHFV causes highly contagious haemorrhagic fever in humans, with a case fatality rate up to 40% [21]. It is transmitted by ticks (*Hyalomma* spp.) or by direct contact to viraemic humans and animals. A small mammal reservoir for CCHFV has been discussed, but not identified. Ticks are now considered both reservoirs and amplifying hosts [21].

Recently, the zoonotic Langya virus (LayV, family *Paramyxoviridae*) was isolated from febrile human patients and detected in Ussuri white-toothed shrews (*Crocidura lasiura*) and Shantung white-toothed shrews (*Crocidura shantungensis*) in China [22]. Gamak virus (GamV) and Daeryong virus have been identified in *C. lasiura* and *C. shantungensis* in Asia, respectively [23]. Recent studies in Belgium identified Melian virus (MeliV) in African large-headed shrews (*Crocidura grandiceps*), Denwin virus (DewV) in European *C. russula* and Ninorex virus in the Eurasian pygmy shrew (*Sorex minutus*), a red-toothed shrew species [24, 25]. Classification of the shrew-associated paramyxoviruses as a separate genus *Parahenipavirus* was proposed and accepted by the International Committee on Taxonomy of Viruses (ICTV) [26]. Parahenipaviruses are phylogenetically related to the members of the genus *Henipavirus*, which includes the highly contagious and lethal zoonotic Hendra virus (HeV) and Nipah virus (NiV) discovered in fruit bats in Australia and Southeast Asia, respectively [27, 28].

Here, we investigated the virome of four white-toothed shrew species present in Europe (total *n*=45) using a straightforward sample-pooling approach, followed by high-throughput RNA sequencing and specific reverse transcription quantitative PCR (RT-qPCR) confirmation and determination of the viral tissue distribution to indicate potential transmission routes. Our study is thus one of the first to record specifically on the virome of white-toothed shrews in Europe. The surprisingly high number of novel viruses suggests a previously underestimated reservoir function of shrews, which might be even greater than that of sympatric rodent and bat species as recently postulated [29], and this is not only in subtropical but also in temperate regions.

METHODS

Sample selection and RNA extraction

A total of 19 bicolored white-toothed shrews (*C. leucodon*), 16 greater white-toothed shrews (*C. russula*), and 6 lesser white-toothed shrews (*C. suaveolens*) covering the known distribution of these species in Germany, captured between 2002 and 2021, and two additional *C. leucodon* collected in the Czech Republic in 2007 were selected. In addition, two Etruscan shrews (*S. etruscus*) from a German breeding colony were included (Fig. S1 and Table S1, available in the online Supplementary Material 1 and Supplementary Material 2). Identification of the shrew species was based on molecular analysis of the *cytochrome b* gene as described previously [30].

First, organ tissues were pooled per individual, consisting of small pieces of brain, lung, spleen, liver and kidney tissues, as available. These tissue pools were directly immersed in 1 ml QIAzol (Qiagen, Germany) and stored at −80 °C until further processing. In addition, intestine tissue samples containing ingesta from several individuals of the same species were pooled and processed according to the individual tissue pools (Table S1).

Tissue pools were homogenized for 2 min at 30 Hz using 5 mm steel beads on a TissueLyser II instrument (Qiagen, Germany). Chloroform (Carl Roth, Germany) was added to each reaction, mixed vigorously and centrifuged at 13,000×g for 10 min. The upper aqueous phase was further processed for total RNA extraction using the Agencourt RNAdvance Tissue Kit (Beckman Coulter, Germany) on a KingFisher Flex Purification System (Thermo Fisher Scientific, Germany) according to the manufacturer's instructions.

Library preparation from RNA and high-throughput sequencing

Total RNA quantity was measured using a NanoDrop ND1000 UV spectrophotometer (Peqlab, Germany), and total RNA quality was assessed using a 4150 TapeStation system (Agilent, Germany). In an attempt to reduce the amount of host-derived ribosomal RNA (rRNA), total RNA was treated with the 'pan mammalia' riboPOOL ribosomal depletion kit (siTOOLs Biotech, Germany) according to the manufacturer's instructions. The rRNA-depleted total RNA was then used for library preparation using the Collibri Stranded RNA Library Prep Kit for Illumina Systems (Invitrogen, Germany) according to the manufacturer's instructions. Final libraries were quantified using a Qubit 2.0 fluorometer in conjunction with the Qubit dsDNA HS Assay-Kit (Invitrogen, Germany). The libraries were then pooled, submitted to CeGaT GmbH (Germany) and sequenced on a NovaSeq 6000 system (Illumina, USA) in 1×100 base pair (bp) mode.

Capture enrichment of high-throughput sequencing libraries

The high-throughput sequencing libraries were further enriched for specific sequences of epizootic and zoonotic viruses using biotinylated RNA baits (VirBaits panel) [31]. In the present study, we used the extended VirBaits 2.0, which contains oligonucleotide baits for the viruses as described by Wylezich *et al.* [31] supplemented with baits for hepatovirus A, hepatitis B virus, hepacivirus C, orthohepevirus A, infectious pancreatic necrosis virus, Zika virus, Usutu virus, Japanese encephalitis virus, yellow fever virus, Kyasanur forest disease virus, tick-borne encephalitis virus, dengue virus, enterovirus C, measles virus, mumps virus, salmonid novirhabdovirus, viral haemorrhagic septicemia virus, Marburg virus, Sindbis virus, chikungunya virus, rubella virus, rustrela virus, La Crosse virus, Lassa mammarenavirus, orthohantaviruses and betacoronaviruses (VirBaits 2.0 one health panel). The VirBaits 2.0 collection comprises 134,710 RNA baits, each with a length of 80 nucleotides. The VirBaits 2.0 panel was implemented following the guidelines provided by the manufacturer (standard protocol of the myBaits manual v.5.00, Arbor Biosciences, September 2020) for 24 h at 60 °C.

The enriched libraries were finally amplified using the Collibri Library Amplification Master Mix (Invitrogen, Germany, 14 cycles) and sequenced on an iSeq system (Illumina, USA) in 2×150 bp mode.

Sequence data analysis

Raw reads were first trimmed for adapter contamination and poor quality using Trim Galore (version 0.6.10) in automatic adapter detection mode. Subsequently, host-specific background was then removed from the trimmed libraries using BBMap (version 39.01, *k*=13 [32]) together with the combined genomic assemblies of *Crocidura indochinensis* (Indochinese white-toothed shrew, GCA_004027635.1), *S. etruscus* (GCF_024139225.1), *Sorex fumeus* (smokey shrew, GCA_026122425.1), *Sorex araneus* (common shrew, GCF_000181275.1) and *Cryptotis parvus* (North American least shrew, GCA_021461705.1) as reference. In addition, rRNA-derived reads were removed using SortMeRNA (version 4.3.6 [33]) with all rRNA entries of the SILVA database (release 138.1 [34]) belonging to the taxon 'Vertebrata' as reference.

The trimmed and host sequence-depleted libraries were individually assembled *de novo* using rnaSPAdes (version 3.15.5 [35]). The metatranscriptomic pipeline SqueezeMeta (version 1.6.2 [36]) was also used for *de novo* assembly, taxonomic classification and quantification. Specifically, SqueezeMeta was run with the option '-contiglen 400' in 'seqmerge' mode, which merges individual assemblies into a single combined assembly prior to further processing. The assembly was then trimmed with regard to poly(A) and poly(T) sequences at the end or start of the contigs, using cutadapt (version 4.0 [37]). This step prevents unspecific mapping to poly(A)-tails. The trimmed *de novo* assembled contigs were then used for a final run of SqueezeMeta using the '-extassembly' option.

Selection of complete viral genomes

Contigs that were classified as viral sequences and likely represented full genomes were selected based on their size from the SqueezeMeta assembly and compared with the rnaSPAdes assembly. For the final quality check, the raw reads were mapped to the likely full genomes using the Geneious Prime (version 2021.0.1) generic mapper. Open reading frame (ORF) annotation was done in Geneious Prime using appropriate references and the 'Find ORFs' function. For selected samples, we re-sequenced tissue pool-derived libraries and analysed them together with sequences obtained from individual kidney samples in order to improve the coverage of the identified genomes.

Sequencing of viral RNA 5′ends

SuperScript III reverse transcriptase (Invitrogen, Germany) and virus-specific primers were used to generate cDNA from the 5′ end of selected virus genomes. The cDNA was then further amplified using the 5′ Rapid Amplification of cDNA Ends (RACE) 2.0 system (Invitrogen, Germany). Final PCR products were prepared for sequencing using BigDye Terminator v1.1 (Applied Biosystems, USA) and sequenced on a 3500 Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analysis of complete viral genomes

Viral sequences were aligned with publicly available reference sequences using MUSCLE (version 3.8.425). Maximum-likelihood phylogenetic trees were calculated using IQ-TREE2 (version 2.2.2.6 [38]) with an automated model selection and each 100 000 ultra-fast bootstrap [39] and SH-aLRT [40] replicates.

In detail, for hepevirus phylogeny, we selected 36 representative genomes of the subfamily *Orthohepevirinae* and five genomes of fish hepeviruses (subfamily *Parahepevirinae*) as references for phylogenetic analysis. The first 450 amino acid (aa) residues of the ORF1-encoded non-structural polyprotein wasere aligned and used for phylogeny.

For paramyxovirus phylogeny, we selected 54 representative genomes of the subfamily *Orthoparamyxovirinae* and one genome of the subfamily *Metaparamyxovirinae* as references for phylogenetic analysis. The aa sequences of the large protein (L, including RNA-directed RNA polymerase, capping and cap methylation activities) were aligned and used for phylogeny.

For nairovirus phylogeny, we selected 46 representative genomes of the genus *Orthonairovirus* and one genome of the genus *Shaspivirus* as references for phylogenetic analysis. The aa sequences of the large protein (L, large segment, containing an RNA-directed RNA polymerase domain) were aligned and used for phylogeny.

For bornavirus phylogeny, we selected 74 shrew and domestic animal-derived genomes of BoDV-1. Borna disease virus 2 (BoDV-2) was used as the outgroup. Nucleotide sequences spanning the nucleoprotein (N), phospho- (P) and X protein-coding sequences were aligned and used for phylogenetic analysis.

Virus-specific RT-qPCR

Primers and probes for RT-qPCR detection of viral RNA of the detected nairo-, paramyxo- and hepeviruses were designed using Primer3web (version 4.1.0 [41]). The L ORF was targeted for nairoviruses and paramyxoviruses and ORF3 for hepeviruses. For specific detection of BoDV-1, the BoDV-1-Mix1-FAM assay was used [42]. A set of primers and probes targeting the ß-actin-2 gene was used as an internal control [43]. Primer and probe sequences are shown in Table S3. The RT-qPCRs were performed using the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, USA) according to the manufacturer's instructions and run on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Germany) with the following protocol: 10 min at 45 °C for reverse transcription, 10 min at 95 °C for polymerase activation, 42 cycles of 15 s at 95 °C, 20 s at 57 °C (with fluorescence detection during this step) and 30 s at 72 °C.

Tissue distribution of novel viruses

An organ tissue panel was prepared from selected animals to assess the tissue distribution of viral RNA. Approximately 50 mg of tissue was homogenized in 500 µl phosphate-buffered saline (PBS) for 2 min at 30 Hz using 5 mm steel beads on a TissueLyser II instrument (Qiagen, Germany). Total nucleic acids were extracted using the NucleoMag Vet Kit (Macherey and Nagel, Germany) on a KingFisher Flex Purification System (Thermo Fisher Scientific, Germany) according to the manufacturer's instructions.

Virus isolation in cell culture

For cell culture isolation of Rasenna virus from *S. etruscus*, organ tissue material was lysed in a cell culture medium and used to inoculate Vero cells (CCLV-RIE 0228) or baby hamster kidney (BHK) 21 cells (CCLV-RIE 0179) in a TC12.5 format (serum-free cell culture medium plus antibiotics). The cell culture supernatant from each cell culture flask was used for passaging to achieve four consecutive passages. In addition, the cells were passaged again separately to obtain four consecutive passages. Tissues used for the different isolation attempts included the liver, spleen, heart, muscle, fat, skin and thoracic and cervical spinal cord.

RESULTS AND DISCUSSION

Overall virome analysis

Metagenomic analysis of tissue and intestine pools from a selected subpopulation of 45 white-toothed shrews collected across Europe (Fig. S1 and Table S1) revealed the presence of a wide range of RNA viruses belonging to the orders *Bunyavirales*, *Mononegavirales*, *Hepelivirales*, *Picornavirales* and *Stellavirales* (Fig. 2). Sequence reads of the orders *Bunyavirales* and *Mononegavirales* were the most abundant ones in the individual-based organ pools, while *Picornavirales* and *Stellavirales* were predominantly detected in the species-based intestine pools. Tissue pools provide the benefit of reduced sampling and sequencing bias due to non-homogeneous virus distribution in the different tissues. After initial sequencing, the libraries were further enriched for a broad range of epizootic and zoonotic viruses using capture enrichment with the VirBaits 2.0 myBait panel (Wylezich *et al.*, unpublished).

Subsequent analysis focused on virus genera with public health implications [1, 7]. In particular, we identified the whole genome sequences of novel orthoparamyxo-, orthonairo- and orthohepeviruses, as well as several complete genome sequences of the zoonotic BoDV-1 and ERVEV [14, 18]. Virus-specific RT-qPCRs were designed in order to determine viral RNA tissue distribution. Based on the observed tissue distribution, kidney tissue from selected individuals was additionally sequenced and included in the analysis. The following sections summarize the results for each virus family.

Detection and analysis of novel paramyxoviruses

Within the family *Paramyxoviridae* (order *Mononegavirales*), there are currently 4 subfamilies with 14 genera established [44]. The subfamily *Orthoparamyxovirinae* comprises several viruses with high impact on human and animal health, such as members of the genera *Morbillivirus* (measles virus and rinderpest virus, the first successfully eradicated epizootic disease) and *Henipavirus* (NiV, HeV), with reoccurring outbreaks of NiV, demonstrating dramatic case fatality rates of 40–70%, including possible human-to-human transmission [1, 28, 45].

Within the tissue pools, we identified genomes (Fig. 3a) of diverse orthoparamyxoviruses that phylogenetically clustered within the newly established genus *Parahenipavirus* (Fig. 3b), extending this shrew-dominated group (Fig. 3b, c) [26, 46]. The novel Hasua virus (HasV) was identified in *C. suaveolens* (KS21-0087) from north-eastern Germany and was phylogenetically closely related to LayV and Mòjiāng virus (MojV). When comparing the aa sequence of the large protein (L) of HasV to LayV and MojV, the identity ranged between 81.0 and 81.5 % (Fig. S2).

Interestingly, we found sequences of another novel orthoparamyxovirus, tentatively named Resua virus (ResV), in the same specimen (KS21-0087), suggesting co-infection. ResV was furthermore identified in two additional *C. suaveolens* from Germany (KS19-0490 and KS20-3619). ResV phylogenetically clustered with a distant group of exclusively shrew-derived paramyxoviruses, such as GamV. Lechcodon virus (LechV) was detected in two *C. leucodon* from southern Germany (KS21-0502, KS21-0453) and grouped basal to HasV and LayV (Figs 3c and S2).

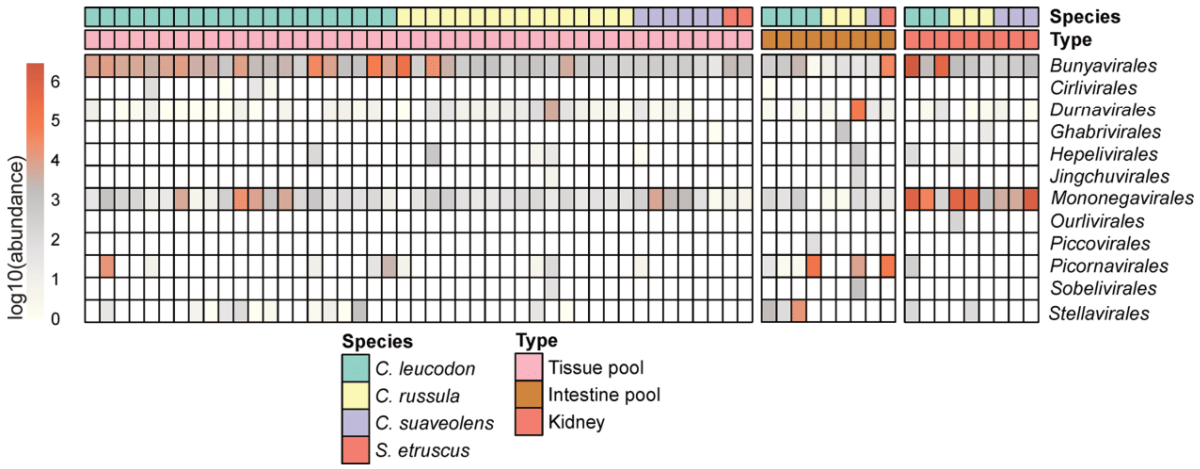

Fig. 2. Viral diversity in different samples from white-toothed shrews. The heatmap shows the relative abundance of viral sequences sorted taxonomically by viral order. Note the abundance of sequence reads of the orders *Bunyavirales*, *Mononegavirales*, *Hepelivirales*, *Picornavirales* and *Stellavirales*. *Bunyavirales* and *Mononegavirales* were the most abundant orders in the tissue pools, while reads of *Picornavirales* and *Stellavirales* were predominantly detected in the intestine pools. Based on the observed tissue distribution, kidney tissue from selected individuals was additionally sequenced and included in the analysis.

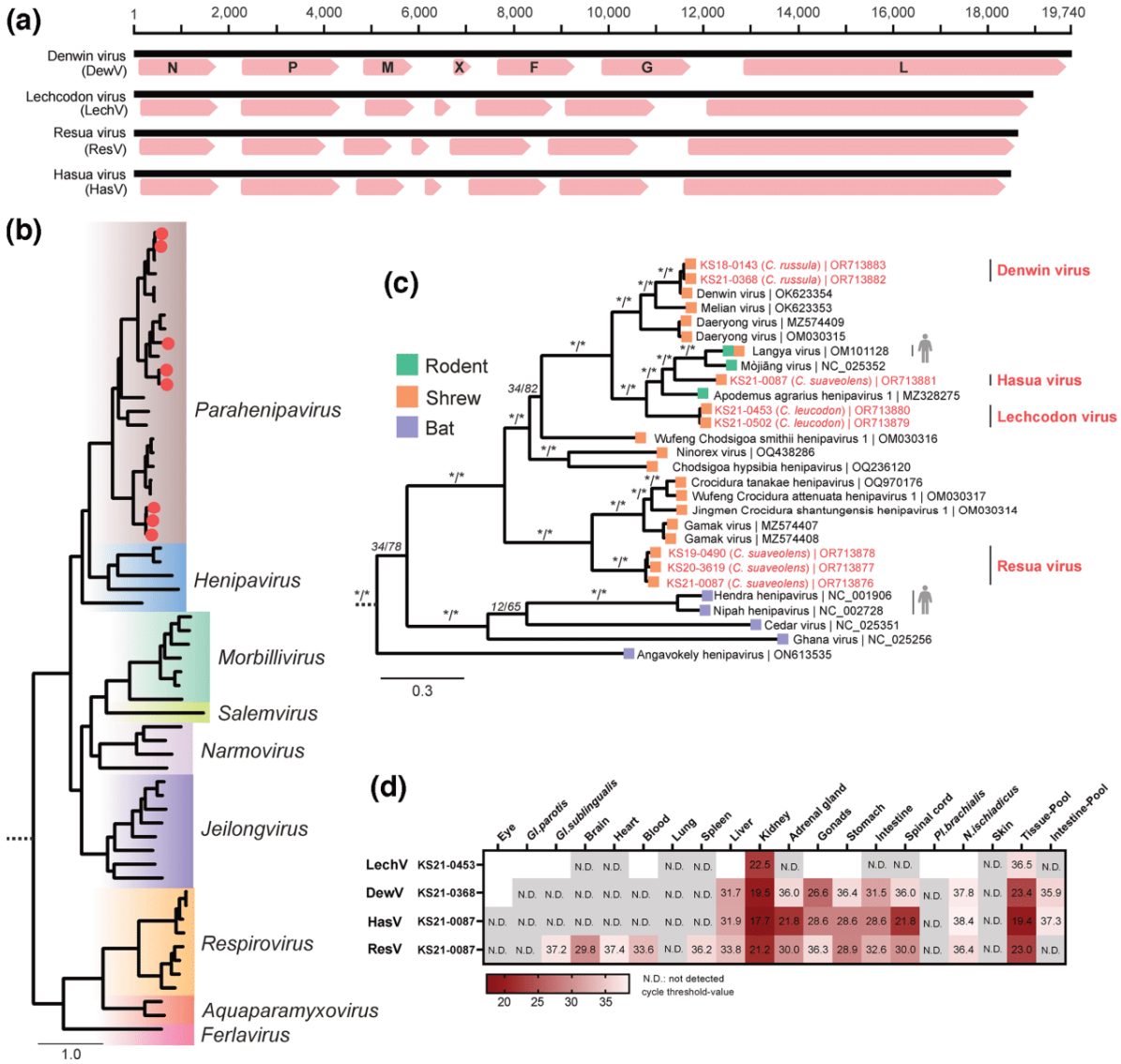

Fig. 3. Detection and analysis of shrew associated paramyxoviruses. (a) The genome structure of the novel paramyxoviruses was similar to that of Denwin virus, with the presence of the hypothetical open reading frame 'X', specific to other shrew-derived paramyxoviruses. (b) For phylogenetic analysis, we selected 54 representative genomes from the *Orthoparamyxovirinae* subfamily, using *Metaparamyxovirinae* as an outgroup. The amino acid sequences of the large protein (L, including RNA-directed RNA polymerase, capping and cap methylation activities) were aligned and used for phylogeny (IQ-TREE2; version 2.2.2.6). (c) Phylogenetic analysis of the genera *Henipavirus* and *Parahenipavirus*. Novel whole genomes are highlighted in red, and host-association is indicated by tip colour. Viruses with described zoonotic potential are highlighted with a human silhouette. Statistical support is shown for main branches using the format [SH-aLRT (%)/ultrafast bootstrap (%)]. Asterisks indicate statistical support ≥80 % and ≥ 95 % for ultrafast bootstrap and SH-aLRT, respectively. (d) Tissue distribution of paramyxovirus RNA using RT-qPCR specific for the L gene region. Results are given in cycle threshold values. No tissue was available for blank fields.

Finally, sequences of the previously described DewV were detected in two *C. russula* (KS18-0143, KS21-0368), demonstrating its presence in Germany. In total, 9 out of 16 *C. russula* (56%, 95% confidence interval (CI): 33–77) were positive for DewV by RT-qPCR, indicating a high prevalence and wide geographical distribution of this virus (Table S1).

Virus-specific RT-qPCR confirmed the presence of these viruses, and viral RNA tropism was assessed, with high levels of viral RNA observed, particularly in kidney tissue. Although further assessment is needed, potential excretion and transmission via urine should be considered when establishing preventive measures (Fig. 3d). Efficient transmission via urine was demonstrated for HeV and NiV, even allowing direct bat-to-human transmission for NiV through the consumption of urine-contaminated

food [28]. Otherwise, transmission of HeV and NiV from their fruit bat reservoir to humans requires an intermediate host, either horses or pigs, respectively [28].

The zoonotic potential of these novel paramyxoviruses cannot be addressed in this study, as further *in vitro* and *in vivo* downstream characterizations are required [6]. However, their phylogenetic proximity to known zoonotic agents (e.g. LayV) and to viruses that, at least experimentally, have the ability to infect human cells (e.g. GamV) [23, 26] clearly warrants such work. These newly identified paramyxoviruses confirm the presence of a phylogenetically related group of shrew-derived viruses that form a sister clade to the bat-borne henipaviruses and support the increasing number of globally distributed paramyxoviruses [23–25, 47].

Detection and analysis of novel nairoviruses

The genus *Orthonairovirus* belongs to the family *Nairoviridae* of the order *Bunyavirales*. Orthonairoviruses are arthropod-borne, globally distributed viruses with a wide range of hosts, including mammals, birds and reptiles. In some cases, they can cause severe or fatal disease in livestock and wildlife, with substantial economic and ecological implications [3, 20]. The reservoir species for many of these viruses have not been successfully identified yet, and small mammals have been considered putative reservoirs or amplification hosts [21]. Orthonairoviruses possess a negative-sense trisegmented RNA genome with a small (S) segment encoding the nucleoprotein, the medium segment encoding the glycoprotein precursor and the large segment encoding the large protein (L) which mediates viral replication and transcription [48].

In the sampled shrew tissue pools, orthonairovirus-related sequences were highly abundant and detected in 10 out of 45 tissue pools (22.2%) across *C. leucodon, C. russula* and *S. etruscus*, but were absent in the analysed *C. suaveolens*.

Several phylogenetically distinct complete sequences of viral genome segments could be deduced (Figs 2 and 4a) and phylogenetically grouped within the Thiafora virus genogroup, which includes the shrew-borne ERVEV, TFAV and CENV (Fig. 4b).

In detail, six whole genomes of the novel Regana virus (REGV) were identified exclusively in *C. leucodon* (KS19-0440, KS20-0043, KS20-0407, KS20-1367, KS21-0453 and KS22-2124) from all over Germany and the Czech Republic. REGV forms a monophyletic cluster basal to the known viruses within the Thiafora virus genogroup (Fig. 4c). Furthermore, several segments of ERVEV were identified in two tissue pools from *C. russula* (KS12-1272 and KS17-1734), and the novel Rasenna virus (RASV) was identified in captive *S. etruscus* (FP20-1), with its sequences clustering between REGV and CENV (Fig. 4c).

The aa sequence similarity of the L-protein between the identified REGV and ERVEV strains ranged between 84.4–99.3% and 79.6–90.2% (Fig. S3), respectively, which is below the ICTV species delimitation threshold of <93% for members of different species in the genus *Orthonairovirus* [48]. However, because of their geographic proximity and common host species, we tentatively considered all of these viruses to be variants of REGV or ERVEV, rather than proposing them as unique viruses.

The presence of multiple ERVEV variants within a single animal was subsequently confirmed using genome-specific RT-qPCR assays (Fig. 4d). This finding indicated the potential for reassortment, which is a process that can result in high genetic variability facilitating host adaptation or host species switches, commonly seen in segmented viruses such as those of the order *Bunyavirales* [21, 49].

Virus-specific RT-qPCR analyses confirmed the presence of the new virus genomes in the tissue pools and in individual tissues, suggesting a broad tissue distribution with high viral RNA loads, especially in well-perfused organs. Liver tissue yielded the lowest cycle threshold values in all individuals (Fig. 4d). These findings are in accordance with a viraemic status of orthonairovirus infection and may indicate its circulation in the bloodstream. The putative role of ticks in the transmission of the orthonairoviruses detected remains a question for further study. However, the presence of RASV in captive *S. etruscus* from a well-established breeding colony suggests arthropod-independent transmission, as these animals were kept in a controlled ectoparasite-free environment [50]. Vertical and efficient direct shrew-to-shrew transmission via scratching and biting during territorial fights may be assumed for the stable viral persistence in the colony and the wild [8]. Isolation of these viruses may improve the knowledge of virus transmission, but attempts to isolate RASV in Vero and BHK-21 cells have not been successful.

The zoonotic potential of these novel viruses is currently unknown; however, ERVEV has been associated with reports of thunderclap headaches in humans [18, 51]. The presence of genetically diverse ERVEV and the identification of new shrew-borne orthonairoviruses (REGV in *C. leucodon* and RASV in *S. etruscus*) demonstrate the high diversity of orthonairoviruses in white-toothed shrews.

Detection and analysis of a novel hepevirus

Orthohepeviruses infect a wide range of species, including humans, pigs, rabbits, rodents, bats and birds. Generally, they are highly host-specific with the exception of zoonotic viruses of the genus *Paslahepevirus* [52]. Human hepatitis E virus (HEV; species *Paslahepevirus balayani*) can be transmitted faecal-orally through contaminated water and the consumption of undercooked meat products. It is a major cause of self-limiting acute hepatitis in humans, but can also induce severe chronic hepatitis in immunocompromised patients [53].

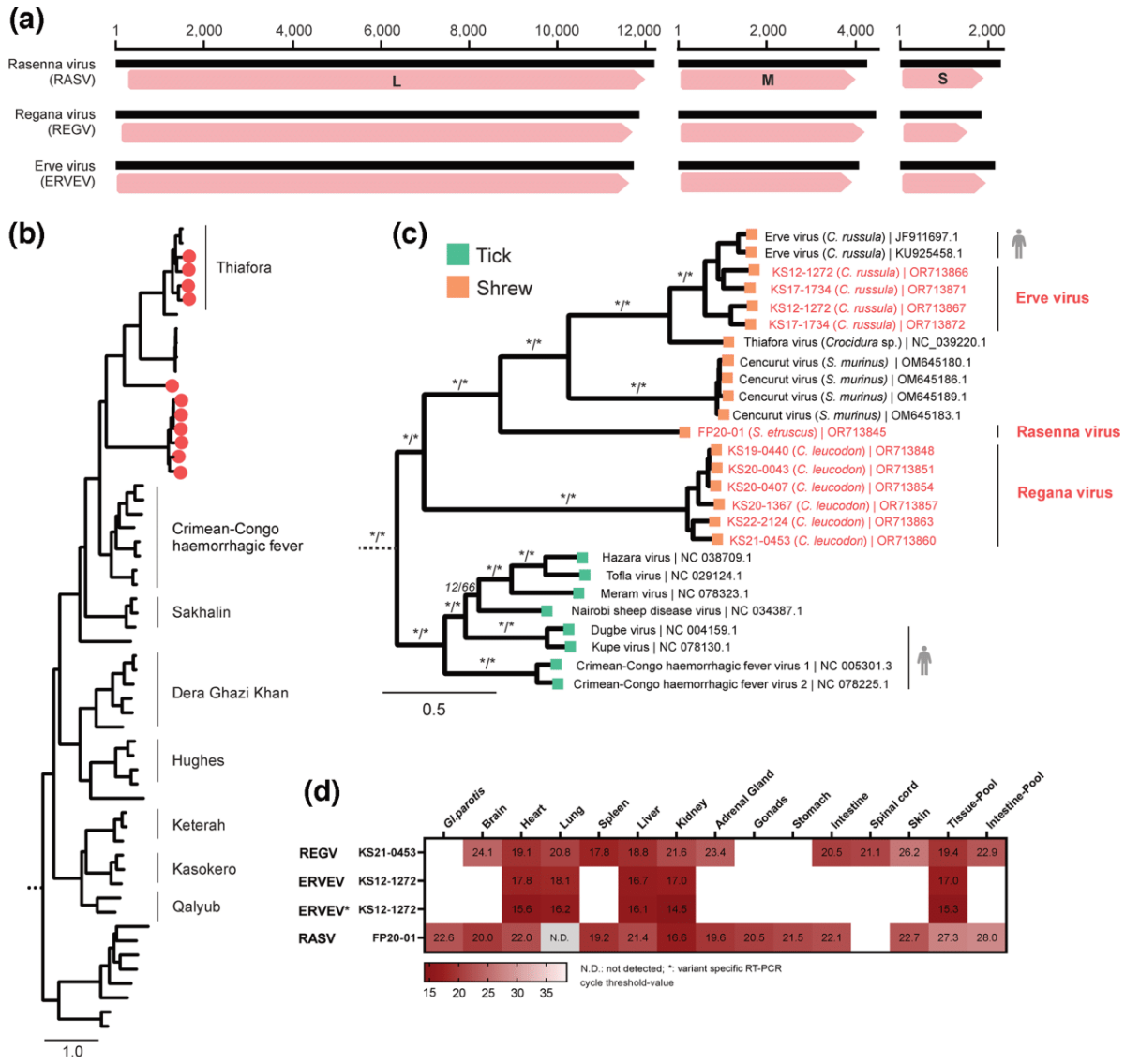

Fig. 4. Detection and analysis of shrew-associated orthonairoviruses. (a) The segmented genome of the novel orthonairoviruses matched the size of the genomes of other members of the family *Nairoviridae*: the small (S) segment encoding for the nucleoprotein and the non-structural NSs, the medium (M) segment encoding for the glycoprotein precursor and the large (L) segment encoding for the RNA-directed RNA polymerase. (b) For the phylogeny of orthonairoviruses, we selected 46 representative genomes of the genus *Orthonairovirus* and one genome of the genus *Shaspivirus* as outgroup. The amino acid sequences of the RNA-directed RNA polymerase were aligned and used for phylogeny (IQ-TREE2; version 2.2.2.6). Novel genomes are indicated as red dots. (c) Detailed view of the phylogenetic relationships within the Crimean-Congo haemorrhagic fever and Thiafora virus genogroups. Newly generated whole genomes of Erve virus, Rasenna virus and Regana virus are shown in red. Host association is indicated by colour. Viruses with described zoonotic potential are highlighted with a human silhouette. Statistical support is shown for main branches using the format [SH-aLRT (%)/ultrafast bootstrap (%)]. Asterisks indicate statistical support ≥80% and ≥ 95% for ultrafast bootstrap and SH-aLRT, respectively. (d) Viral RNA tissue distribution as determined by virus-specific RT-qPCRs. KS12-1272 was tested with two different primers and probe sets to differentiate between the two strains of Erve virus. Results are given in cycle-threshold values. No tissue was available for blank fields.

Two closely related whole genomes of a novel hepevirus of the subfamily *Orthohepevirinae* were identified in two specimens of *C. russula* (KS12-1272, KS21-0273) captured in western and eastern Germany (Table S1). They show a genome organization most similar to viruses of the genus *Paslahepevirus*, with an overlapping region of ORF2/ORF3, and the absence of ORF4, an ORF identified in rat hepatitis E virus (Fig. 5a) [54]. This similarity in genome organization is reflected in the phylogenetic position of shrewHEV, which clusters with strains of the genus *Paslahepevirus* well separated from strains of the genus *Rocahepevirus* (Fig. 5b). The aa sequence of the first 450 aa of the ORF1-encoded non-structural polyprotein of the novel shrewHEV showed an identity between 55.7 and 61.9% in comparison to members of the genus *Paslahepevirus* and between 53.1 and 58.4% to members of the

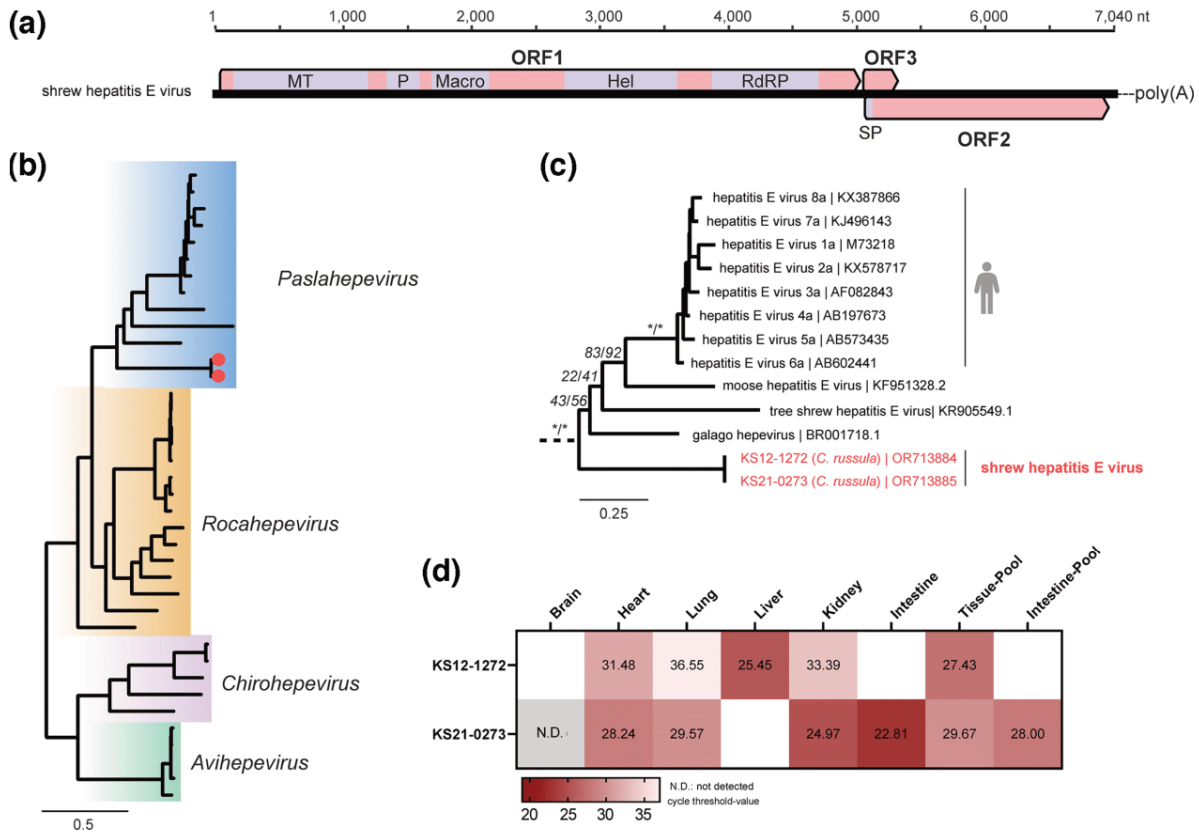

Fig. 5. Detection and analysis of shrew-associated hepevirus. (a) Genome structure of the novel shrew hepatitis E virus. (b) For the phylogenetic analysis of the novel hepevirus, 36 representative genomes of the subfamily *Orthohepevirinae* and five genomes of fish hepeviruses (subfamily *Parahepevirinae*) were selected as references. The first 450 amino acids of ORF1-encoded non-structural polyprotein were aligned, and a phylogenetic tree was calculated (IQ-TREE2; version 2.2.2.6). Novel genomes are indicated as red dots. (c) Detailed view on the phylogenetic relations within the genus *Paslahepevirus*. Viruses with described zoonotic potential are highlighted with a human silhouette. The novel hepevirus sequences are indicated in red. Statistical support is shown for main branches using the format [SH-aLRT (%)/ultrafast bootstrap (%)]. Asterisks indicate statistical support ≥80% and ≥ 95% for ultrafast bootstrap and SH-aLRT, respectively. (d) Viral RNA tissue distribution of the novel shrew hepatitis E virus in two *C. russula* (KS12-1272, KS21-0273), as detected by virus-specific RT-qPCR. Results are given in cycle threshold values. No tissue was available for blank fields.

genus *Rocahepevirus* (Fig. S4). The highest viral RNA loads were detected in the liver tissue of KS12-1272 and in the kidney and intestinal tissue of KS21-0273, suggesting faecal-oral transmission (Fig. 5c).

Detection and analysis of Borna disease virus 1

BoDV-1 belongs to the genus *Orthobornavirus* (family *Bornaviridae*). It causes sporadic but fatal encephalitis in domestic animals, primarily horses, sheep and New World camelids, and was only confirmed as zoonotic in 2018 [14, 42]. The transmission from its reservoir to dead-end hosts, its presence in the reservoir population and the appearance of its endemic area are still poorly understood.

In this study, we generated seven new BoDV-1 complete genome sequences from four *C. leucodon* and, for the first time, from two *C. suaveolens* and one *C. russula* (Fig. S5). In accordance with their geographic origin, these new BoDV-1 sequences fall within the established phylogeographic clusters [14, 55]. The presence of BoDV-1 RNA in the tissue pools was confirmed by specific RT-qPCRs.

Co-infection of different viruses

Several shrews in the study demonstrated co-infections with multiple viruses. For example, *C. russula* KS12-1272 was found to carry three viruses: shrewHEV (complete genome identified), ERVE (two different complete L segments detected) and DewV (detected by RT-qPCR). Similarly, *C. suaveolens* KS21-0087 showed a triple infection containing two distinct paramyxoviruses (HasV and ResV) and BoDV-1. In addition to the complete genome of shrewHEV, DewV was also identified by RT-qPCR in *C.*

russula KS21-0273. *Crocidura russula* KS21-0368 tested positive for both DewV and BoDV-1, while *C. suaveolens* KS20-3619 tested positive for BoDV-1 and ResV. The complete genomes of LechV and REGV were present in *C. leucodon* KS-0453. BoDV-1 and REGV were identified in both *C. leucodon* specimens (KS20-1367 and KS21-0392), as detailed in Fig. S6.

Influence on virus sequencing efficiency using bait-captured libraries

The influence on virus-to-background ratio by application of VirBaits 2.0 capture enrichment was virus-specific. For BoDV-1, the efficiency was highly increased, while for the newly discovered viruses, the virus-to-background ratio was only minimally improved, if at all (Fig. S7). This can be explained as the used capturing probes were tailored to the genetic sequences of known viruses (e.g. CCHFV, HeV, NiV, HEV and BoDV-1), enhancing their binding efficiency and capture performance for those targets. In the case of BoDV-1, the probes are likely to have high affinity and specificity, resulting in a significant increase in the virus-to-background ratio. This means that the probes effectively capture the viral sequences, reducing the amount of non-target, or background, sequences in the library, thereby improving sequencing efficiency. On the other hand, for newly discovered viruses that are highly divergent from the references on the nucleotide level, the probes used in the bait set might be less specific, and efficiency is decreased. This is a common challenge in capture enrichment technologies, where the effectiveness of the probes depends heavily on their sequence complementarity to the target viruses [31].

CONCLUSION

Investigations of species-rich and phylogenetically ancient wildlife taxa such as shrews improve our understanding of global virus distribution. Revisions to existing taxonomy and the continued discovery of new shrew species, as well as the expansion of the range of some shrew species, demonstrate the high complexity of this group of animals. There is limited information available on the basic parameters of shrew's biology, such as population structure and dynamics. However, shrews may share similar properties with other so-called viral hyperreservoirs, such as bats and rodents. Their high metabolism, torpor, fast life cycle and unknown immunological responses to viral infection may enable them to sustain and spread viral infections without developing any disease.

Viruses detected in *C. russula*, which has a North African origin, are genetically similar to other viruses detected in African shrews (ERVEV and TFAV for nairoviruses, and DewV and MeliV for paramyxoviruses), whereas *C. suaveolens*, which is widely distributed across Eurasia, presented viruses with close relatives detected in Asian shrews (HasV and LayV). This suggests a certain degree of co-evolution between the shrew species and their carried viruses.

In the context of increased pandemic preparedness, these viruses and their reservoirs need to be studied in more detail to assess their pathological relevance, mode of transmission and potential as surrogates for vaccine development. Although the elusive behaviour of these synanthropic shrews makes it difficult to grasp the human–shrew interface, it does exist, as evidenced by human BoDV-1 infections. In any case, the findings indicate the need for biosafety considerations when handling wild animals of these species.

Our results demonstrate the great diversity of viruses harboured in wildlife, not only in biodiversity hotspots but also in the human-dominated landscapes of Europe. In a holistic One Health approach, future studies should evaluate the potential influence of anthropogenic land use, biodiversity and climate change on the range of these neglected reservoir species and their potential as reservoirs while acknowledging the conservation of white-toothed shrews.

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Author contributions

Methodology: F.P., D.H. and M.B. Validation: F.P. Data curation: F.P. Investigation: V.C.H., B.L., J.S.-E., C.W., D.H. and F.P. Resources: J.J., M.Ba., M.H. and M.Br. Writing – original draft preparation: V.C.H. and F.P. Writing – review and editing: V.C.H., B.L., J.S.-E., M.Ba., C.W., D.H., J.J., M.H., M.Br., R.G.U., M.B. and F.P.

Visualization: F.P. and V.C.H. Conceptualization, supervision, project administration and funding acquisition: R.G.U., M.B. and F.P. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Ethical statement
Shrews were by-catches of trapping approved by state agencies (permit no: 22-2684-04-15-105/16 (GER-TH), 42502-2-1548 (UniLPZ; GER-ST), 84–02.04.2015.A279 (GER-NW) and V/2/2006/10 (CZ)) and of trapping conducted by forestry authorities within their professional duties. Etruscan shrew tissue was collected according to a permit T0078/16 given to the Brecht group. The majority of small mammals is originated from a Citizen Science-based project (cat prey, found dead); therefore, no further permits were required.

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Publication 3: One Health in action: Investigation of the first detected local cluster of fatal Borna disease virus 1 (BoDV-1) encephalitis, Germany 2022

One Health in action: Investigation of the first detected local cluster of fatal Borna disease virus 1 (BoDV-1) encephalitis, Germany 2022

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Full Length Article

One Health in action: Investigation of the first detected local cluster of fatal borna disease virus 1 (BoDV-1) encephalitis, Germany 2022

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ABSTRACT

Background: Zoonotic Borna disease virus 1 (BoDV-1) causes fatal encephalitis in humans and animals. Subsequent to the detection of two paediatric cases in a Bavarian municipality in Germany within three years, we conducted an interdisciplinary One Health investigation. We aimed to explore seroprevalence in a local human population with a risk for BoDV-1 exposure as well as viral presence in environmental samples from local sites and BoDV-1 prevalence within the local small mammal population and its natural reservoir, the bicoloured white-toothed shrew (*Crocidura leucodon*).

Methods: The municipality's adult residents participated in an anonymised sero-epidemiological study. Potential risk factors and clinical symptoms were assessed by an electronic questionnaire. Small mammals, environmental samples and ticks from the municipality were tested for BoDV-1-RNA. Shrew-derived BoDV-1-sequences together with sequences of the two human cases were phylogenetically analysed.

Results: In total, 679 citizens participated (response: 41 %), of whom 38 % reported shrews in their living environment and 19 % direct shrew contact. No anti-BoDV-1 antibodies were detected in human samples. BoDV-1-RNA was also undetectable in 38 environmental samples and 336 ticks. Of 220 collected shrews, twelve of 40 *C. leucodon* (30%) tested BoDV-1-RNA-positive. BoDV-1-sequences from the previously diagnosed two paediatric patients belonged to two different subclades, that were also present in shrews from the municipality.

Interpretation: Our data support the interpretation that human BoDV-1 infections are rare even in endemic areas and primarily manifest as severe encephalitis. Sequence analysis linked both previous paediatric human infections to the local shrew population, but indicated independent infection sources.

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1. Background

Known as the infectious agent of Borna disease in animals for many decades, it was only proven in 2018 that Borna disease virus 1 (BoDV-1, species *Orthobornavirus bornaense*) also possesses zoonotic potential, causing severe encephalitis in humans with a high case-fatality rate [1,2]. BoDV-1 is an enveloped virus of the family *Bornaviridae* with a negative-stranded, non-segmented RNA genome [3]. The RNA genome encompasses approximately 8.9 kilobases organised in six open-reading frames and encoding six viral proteins: The membrane-bound glycoprotein (G; gp84) is cleaved into two functional proteins by the cellular protease furin; the N-terminal domain (gp56) mediates viral entry by binding to a presently unidentified cellular receptor, while the C-terminal domain (gp43) is responsible for the pH-dependent membrane fusion within endosomes after viral uptake. A viral matrix protein (M; p16) that is associated with the inner membrane is essential for viral assembly and budding. The nucleoprotein (N; isoforms p38 and p40) is expressed most abundantly in infected cells and, together with a phosphoprotein (P; isoforms p19 and p23) and the large protein harbouring the RNA-dependent RNA polymerase (L; p190) belongs to the ribonuclear proteins that are associated with the genomic viral RNA. A small accessory protein X (X; p10) possesses regulatory, immuno-modulatory and anti-apoptotic properties. BoDV-1 replicates within the cell nucleus and leads to persistent infections.

The bicoloured white-toothed shrew (*Crocidura leucodon* (Hermann, 1780)) has been identified as reservoir host for BoDV-1. Virus-carrying shrews excrete the virus via saliva, urine and faeces without showing clinical signs [4,5].

Horses, sheep and New World camelids (alpacas, lamas), but also a number of other mammals including humans, are susceptible to infection with BoDV-1 usually resulting in fatal BoDV-1-induced encephalitis. They act as dead-end hosts without observed viral shedding.

Before 2018, discussions of the zoonotic potential of BoDV-1 were controversial. Studies reporting a substantial seroprevalence of anti-bornavirus antibodies in human populations worldwide, possibly associated with neuropsychiatric disorders, are meanwhile regarded as unspecific findings due to cross-reactivities in the applied serological tests (reviewed in [6,7]). After it was finally proven that BoDV-1 can cause severe encephalitis also in humans [1,2], debates on the seroprevalence and possible association with clinical manifestations other than severe encephalitis have resumed, requesting a re-analysis of these issues following improved diagnostic standards.

Since 2020, approcimately 50 human patients with confirmed BoDV-1 infection have been reported to German health authorities (as of February 2024), both as retrospectively identified and new cases. All patients exhibited symptoms of a severe (non-purulent) encephalitis. After a variable initial phase of non-specific flu-like symptoms and reduced general conditions, the disease progressed rapidly with severe neurological symptoms such as dysphagia, progressive paresis, respiratory insufficiency, somnolence, finally leading to deep irreversible coma and death [8,9]. In humans, detection of BoDV-1-specific RNA (e.g. in cerebrospinal fluid (CSF) or brain tissue) or antigen (e.g. in brain tissue) is classified as a confirmed case, while the presence of severe neurological symptoms of encephalitis/encephalopathy in combination with the detection of anti-bornavirus antibodies are classified as probable cases [9]. The *intra vitam* diagnosis can be challenging as the sensitivity of RNA detection in CSF is limited and detectable seroconversion often occurs only in later stages of the disease [8,10].

Endemic areas for BoDV-1 are located in Eastern and Southern Germany as well as in small parts of Austria, Switzerland and in the Principality of Liechtenstein [7,11]. Since the introduction of mandatory reporting of human bornavirus infections in Germany in March 2020, up to six acute human BoDV-1-encephalitis cases have been reported annually. The earliest retrospectively diagnosed case in the German federal state Bavaria, however, dates back to 1996 [12]. Over 90 % of the known human cases and numerous detections of BoDV-1 infections in domestic mammals and shrews have occurred in Bavaria [13].

Comprehensive studies of small mammal populations in BoDV-1-endemic areas are lacking especially in direct association to human BoDV-1 cases. In particular, it remains unclear how and at which prevalence the virus is maintained in the reservoir population. The geographical range of *C. leucodon* extends from Northern France to the Caspian Sea [14] and is fluctuating on its edges [15,16]. In Bavaria, *C. leucodon* is commonly found in arid, open areas at an altitude up to ~680 m [17] and shows synanthropic behaviour, colonising gardens, sport fields and parks. This shrew may even invade human dwellings, especially during periods of low temperatures outside [17]. The route of BoDV-1-transmission from the reservoir to dead-end hosts, however, remains unclear. The only risk factor identified, so far, was living rurally in a stand-alone location in a virus-endemic area [18]. These circumstances not only make it difficult to develop targeted recommendations for preventing spillover infections, but also lead to a high level of insecurity and fear amongst the human population in areas with proven BoDV-1-occurrence. In addition, indirect transmission via the environment cannot be excluded, as early data suggest that BoDV-1 can remain infectious in sterile liquids at ambient temperatures [19].

In a Bavarian municipality with approximately 2000 inhabitants, two paediatric cases of fatal BoDV-1-encephalitis occurred in 2019 (case 1) and 2022 (case 2), constituting the first known local cluster of this rare disease [20]. We took this cluster as an opportunity for a comprehensive, interdisciplinary investigation applying the One Health approach. Considering human, animal and environmental aspects, we aimed to study the seroprevalence of BoDV-1-reactive IgG antibodies in the local human population. In addition, we investigated the occurrence of BoDV-1 in potential reservoir species, examined the genetic variability of BoDV-1 in the affected region and aimed to identify possible sites of infection (Fig. 1).

2. Methods

2.1. BOSPEK-study

We conducted a sero-epidemiological study ('Study on Clinical Spectrum of Human Infections with BoDV-1', BOSPEK-study) in order to assess the seroprevalence of BoDV-1-reactive IgG antibodies in a defined local human adult population with an assumed risk of BoDV-1 exposure. To enable a *post-hoc* analysis of the association of possibly detected anti-BoDV-1 IgG antibodies with clinical symptoms or risk factors, an electronic questionnaire was included in the anonymous study. We asked for information on potential risk factors, underlying diseases and neurological symptoms (Supplement **Appendix S1a-b**).

All inhabitants of the affected municipality aged ≥18 years (n = 1648; [21]) were invited to participate in the serological testing. From a randomly selected subgroup (n = 23), lithium-heparin blood was taken in order to perform a BoDV-1-specific T cell assay (BoDV-1-ELISpot [22]). All serum samples were examined by both an

immunofluorescence antibody test (IFAT) and a previously published BoDV-1-ELISA system using recombinant viral N, P and X and protein to detect anti-BoDV-1 IgG antibodies [2,8,23,24]. A detailed description of the diagnostic methods used in the BOSPEK-study (BoDV-1-ELISA, BoDV-1-IFAT, BoDV-1-ELISpot) can be found in the Supplement (**Table S1a-c**).

2.2. Investigation of the shrew population

In collaboration with employees of the local public health authority and of the municipality, a citizen science project was established: citizens sent in dead shrews found in the municipality or preyed by cats. Information on the identification of small mammal species (mice vs. shrews) and instructions how to safely collect the shrews applying biosafety measures were provided.

Small mammals were additionally trapped at five localities with 20 snap traps each, during a period of two weeks in September 2022 (Supplement **Figure S10**). Snap traps were baited with a mixture of peanut butter and mealworms to attract insectivores and with raisins and oats to attract insects, which would then be of interest to shrews. The traps were checked twice daily and rebaited. The carcasses were stored and transported in a frozen state.

The species was determined via molecular analysis of the *cytochrome b* gene [25] and brain tissue of the specimens was examined for BoDV-1-RNA by RT-qPCR [2,26] (Supplement **Table S4**). A detailed description of the methods used for this purpose can be found in **Table S1d-e**.

2.3. Phylogenetic analysis of BoDV-1-sequences from the municipality

BoDV-1-sequences covering the N, X and P genes (1824 nucleotides) were generated by Sanger sequencing from all BoDV-1-positive shrew brain samples. For the phylogenetic analysis, a Jukes-Cantor neighbour-joining tree was generated from all sequences generated in this study together with the BoDV-1-sequences of human cases 1 and 2 from the municipality (GenBank accession numbers: MT364324 and OP776335) as well as all available BoDV-1-sequences from domestic mammals, humans and shrews from the known endemic areas. A detailed description of the analysis can be found in **Table S1f.**

2.4. Research on BoDV-1-tenacity

The stability of infectious virus was investigated for BoDV-1-isolate Regensburg 2019 (human brain, GenBank accession number: LR722643 [8]). Briefly, cell-culture supernatant containing $10^{1.4}$ $TCID_{50}$/ml BoDV-1 was stored in Petri dishes (1) for one day under dry conditions, and (2) poured onto garden herbs and leaves and stored under dry and moist conditions for 1–4 days. At different time points, the material was resuspended in sterile medium added to Vero cells, which were subsequently passaged for up to 10 weeks. Successful isolation of the virus was confirmed by detection of viral RNA in cell culture supernatant by RT-qPCR. A detailed description can be found in **Table S1g-h** of the Supplement.

2.5. Environmental investigation

To further investigate the possibility of BoDV-1-transmission via the environment, we conducted environmental sampling at the patients' living environment and locations regularly frequented by them. The samples were processed and analysed as described in the Supplement (**Table S1i-k**).

2.6. Investigation of ticks

Although there has been no evidence of tick-borne BoDV-1-transmission so far, we analysed castor bean ticks (*Ixodes ricinus*) collected at two locations, where the patients had spent some leisure time, for the presence of BoDV-1-RNA by RT-qPCR (Supplement **Table S1l**).

2.7. Statistical analysis

Data collected in the BOSPEK-study were cleaned and analysed descriptively in R version 4.0.2 (Vienna, Austria) (packages tidyverse, lubridate, rio, writexl, janitor). Figures were created using Microsoft Excel and GraphPad Prism version 8.4.2.

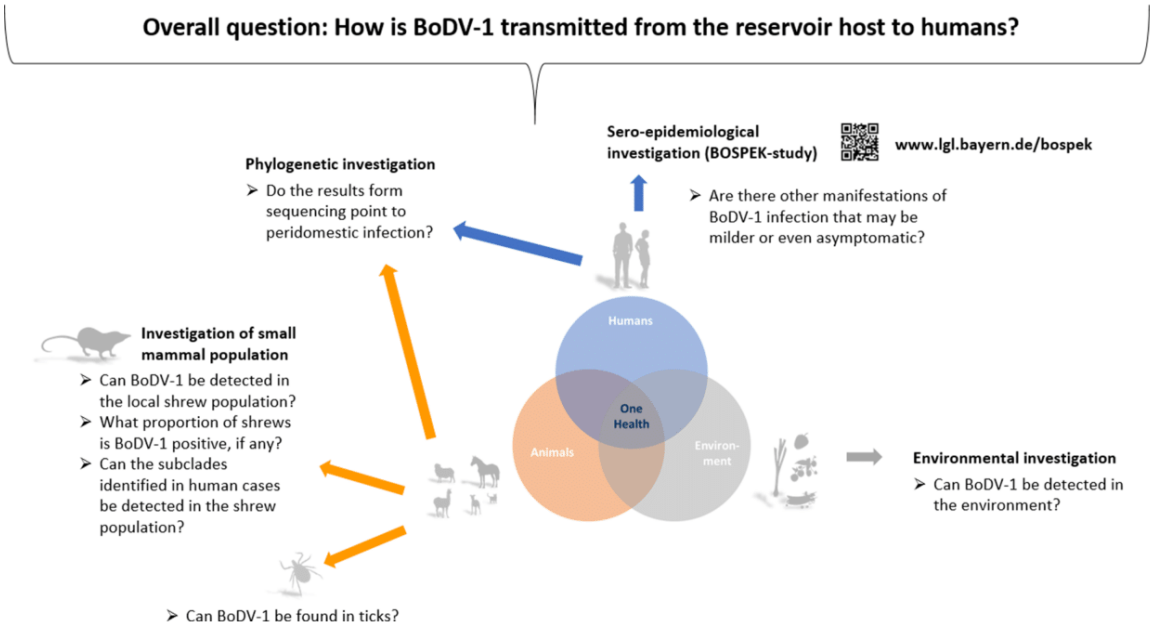

Fig. 1. Overview of the BoDV-1 One Health project 2022: studies conducted and related study questions.

3. Results

3.1. BOSPEK-study

3.1.1. Study population and epidemiological investigation

In total, 679 adult inhabitants participated in the BOSPEK-study (response rate: 41 %), of whom 356 (53 %) were female and 323 (47 %) were male [11](Supplement **Figure S1**). Amongst study participants, 38 % reported to have noticed shrews in their living environment, 19 % reported direct contact with a shrew and 23 % noticed direct contact to the droppings of a shrew or other small mammal (Fig. 2). The proportion of study participants who could correctly differentiate between mice and a shrew from pictures (we showed *Myodes glareolus* and *Apodemus flavicollis*, order Rodentia, and *C. leucodon,* order Eulipotyphla) was high at 93 %. When asked for the frequency of animal contacts, 47 % of participants reported to have "very often" or "often" contact with cats, followed by contact with dogs (22 %) and rabbits/hares (8 %; Supplement **Figure S4**). 96 % of the participants stated spending time outside in nature "very often" or "often" (**Figure S5**). The vast majority of participants lived in a house (91 %; 618/679) and had a garden (99 % of those living in a house). Amongst participants living in a flat, 79 % reported owning a garden. In total, 65 % of the participants' dwellings directly bordered on meadows or pastures, 38 % on fields and 23 % on forests (**Table S2**). An overview of the prevalence of mental disorders and other diseases amongst participants as well as reported neurological symptoms is given in the Supplement (**Table S3, Figures S2** and **S3**). Depression (lifetime prevalence 10 %), anxiety disorders (5 %) and chronic fatigue syndrome (2 %) were reported most frequently (**Table S3**).

3.1.2. Serological testing

In the ELISA, 11.9 % (81/679) of all samples showed reactivity against a single BoDV-1-antigen, while 1.0 % (7/679) were found to have reactivity with two of the tested antigens. None of the sera recognized all three viral antigens. Reactivity against N protein was detected in 5.0 % (34/679), reactivity against X protein in 1.0 % (7/679) and reactivity against P protein in 8.0 % (54/679) of the tested sera. The IFAT, the established gold standard serological test, did not detect a specific nuclear staining pattern in any of the samples. As previously described, the specificity of single antigen reactivities in the ELISA is low, while double antigen reactivity demonstrated a specificity of 97.7 % in previous studies [24]. For further investigation, all seven sera with double antigen reactivity as well as all samples with high reactivity (>2.0 S/CO value) against a single antigen (n =29) in ELISA were sent to two independent diagnostic laboratories for evaluation via IFAT. Both laboratories independently reported negative test results by IFAT. Thus, the ELISA reactions of these 36 samples were interpreted as false positive. Accordingly, no confirmed positive serological result was obtained for any of the 679 participants.

3.1.3. BoDV-1-ELISpot

The subgroup of participants of whom peripheral blood monocytes (PBMC) were evaluated by BoDV-1-ELISpot (n =23) had an age-group and gender distribution comparable to the whole BOSPEK-study population (**Figure S1**). As previously described [22], peptides spanning the whole viral proteins N, X and P were used for stimulation of isolated PBMC. Viability of isolated PBMC was >70 % for all samples. Cut-off values for the BoDV-1-ELISpot >8 spot-forming units (SFU) for stimulation with N-derived peptides, >3 SFU for stimulation with X-derived peptides and >2 SFU for stimulation with P-derived peptides have previously been derived, and result in a specificity of 89 % (N), 100 % (X) and 93 % (P) in a validation cohort of patients after kidney transplantation [22]. In the tested BOSPEK-subgroup, 21 samples were below these cut-off values for all stimulation conditions.

One sample showed 9 SFU by stimulation with N- and 3 SFU by stimulation with P-derived peptides, resulting in the interpretation as a borderline test result together with another sample, which showed 6 SFU by stimulation with P-derived peptides (SFU for N and X below cut-off value). All previously tested patients with confirmed BoDV-1-infection had shown positive results for stimulation with N-derived peptides [22]. These findings are in line with the expected proportions of unspecific reactions, based on the previously established specificities of the assay [22]. Corresponding sera of the two participants did not show any reactivity against any viral antigen in the IgG-ELISA and were negative in the IFAT, further supporting the interpretation as unspecific.

3.2. BoDV-1-tenacity & environmental investigation

In initial studies on viral tenacity, we examined the stability of infectious BoDV-1 to desiccation in Petri dishes (Supplement **Figure S6A**) and on garden herbs and leaves (**Figure S6B**). For this purpose, a stock solution containing $10^{1.4}$ $TCID_{50}$/ml of BoDV-1-isolate Regensburg 2019 was used. Detection of BoDV-1-RNA in cell culture supernatants after up to 10 weeks of passaging demonstrated that BoDV-1 remained infectious over 1 to 4 days of desiccation at ambient temperatures (**Figure S6C**).

Furthermore, we tried to determine whether viruses can be recovered from environmental samples. In a pilot study at a village in the mideast of Bavaria, where BoDV-1-infected shrews had previously been

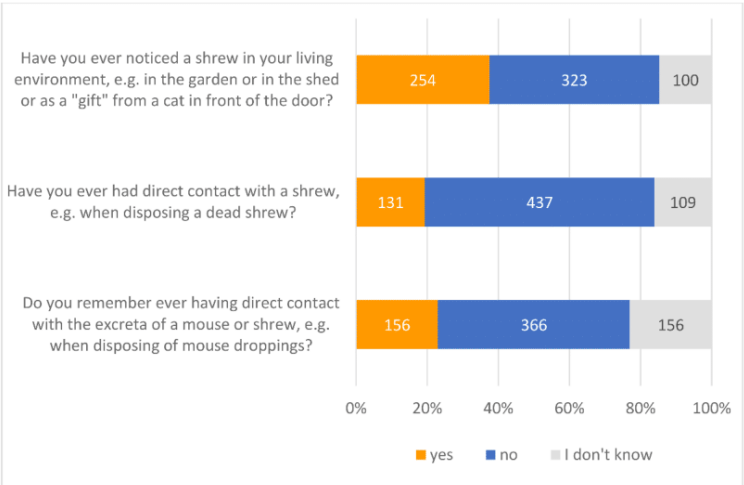

Fig. 2. Reported indirect and direct exposure to shrews and shrew excreta amongst participants of the BOSPEK-study (n = 679).

identified [11], we collected soil and grass from three sites in separate plastic bags (**Figure S7A**). After three days, the cell culture inoculated with a sample from the edge of a small mammal burrow detached from the flask (**Figure S7B**). The virus causing the cytopathic effect could be transferred three times to uninfected Vero cells and once to MRC-5 fibroblasts using filtered fluids. Next-generation sequencing identified a mammalian orthoreovirus. Thus, demonstrating that this method can be used to recover infectious virus from environmental samples.

A similar approach was applied in the studied municipality in order to identify sites posing a risk of infection from environmental sources. The sites for environmental sampling were selected according to the criteria of i) accessibility for children, ii) presence of small mammal activities (e.g. small mammal burrows, droppings), iii) indications from the public about sightings of small mammals, and iv) suitable habitats for shrews. 38 environmental samples were recovered from public localities, including the kindergarten (n =7), children's playground (n =4), two soccer fields (n =12), and surroundings of a small marsh (n =5) (**Figure S8**). In addition, five samples each were taken from the patients' living environments.

None of the environmental samples contained BoDV-1-RNA, whereas the bacteriophage MS2 spike control was amplified as expected, ruling out RT-PCR-inhibiting factors (**Figure S9A**). To ensure that low BoDV-1-levels could have been detected in the environmental samples, a BoDV-1 stock solution containing $10^{4.9}$ BoDV-1-RNA copies/ml was subsequently added to all bags at a final dilution of 1:20 to 1:100. BoDV-1-RNA was detected in 26 of 38 spiked samples (68.4 %), although viral loads were slightly lower than the expected ($10^{2.9}$ to $10^{3.6}$ RNA copies/ml). Supernatants of Vero cells inoculated with the fluids from the original samples remained negative until week 10 (**Figure S9B**), as did the cells at the end of the cultivation period. In contrast, the positive control culture inoculated with the BoDV-1 stock solution ($10^{1.4}$ $TCID_{50}$/ml) became positive as of week 3 (**Figure S9B**). None of the cell cultures inoculated with the environmental samples showed contamination with bacteria or fungi. Overall, neither BoDV-1-RNA nor infectious virus were recovered from environmental samples.

3.3. Investigation of ticks

336 *Ixodes ricinus*-ticks were collected from two locations in the municipality. All ticks tested negative for BoDV-1-RNA.

3.4. Small mammal investigations

Between July 2022 and December 2023, 269 small mammals were collected across the municipality and investigated for the presence of BoDV-1-RNA. In total, 231 (86 %) of the small mammals were cat preys or were found dead for unknown reasons and originated from the citizen science approach. Only the minority of investigated specimens (38 animals; 14 %) were collected during the two-week trapping period of this study (**Figure S10**). 220 of the collected animals were shrews, representing 4 of the 9 shrew species known to be endemic in Germany [17]. The remaining 49 animals belonged to four different rodent species. *Sorex araneus* represented the majority (n =114; 52 %) of the analysed shrews, followed by *C. leucodon* (n =40; 18 %), *Neomys anomalus* (n =37; 17 %) and Sorex minutus (n =29; 13 %; **Table S4**). BoDV-1-RNA could be detected in brain samples from 12 of the 40 *C. leucodon* (30 %, 95 % confidence interval: 18 % - 45.5 %). All BoDV-1-positive *C. leucodon* were preyed by cats or found dead, therefore no precise location of their territories prior to death was available. One *C. leucodon* was collected during the trapping period, but tested negative for BoDV-1-RNA. Neither BoDV-1-RNA nor RNA of other orthobornaviruses was detected in any of the other investigated species.

3.5. Phylogenetic investigation

Phylogenetic analysis was performed for partial BoDV-1-genome sequences generated from all BoDV-1-infected shrews together with the previously published sequences from both human patients (case 1 and 2) and all publicly available sequences originating from shrews, domestic mammals and humans in the BoDV-1-endemic area (Fig. 3). The analysis revealed all BoDV-1-sequences from shrews and from both human cases from the municipality to belong to BoDV-1-subcluster 1A (Fig. 3), which is known to be endemic in south-eastern and south-western Bavaria and eastern parts of Baden-Wuerttemberg [8,27]. As described previously [20], the BoDV-1-sequences of cases 1 and 2 (MT364324 and OP776335, respectively) were not identical. They shared 99.1 % pairwise nucleotide (nt) sequence identity and belonged to separate subclades within cluster 1A. The clade of case 1 was composed mainly of sequences originating from domestic mammals and humans from neighbouring districts to the east of the municipality, while the clade of case 2 harboured sequences from neighbouring districts to the west [11]. Of the twelve shrew-derived BoDV-1-sequences from the municipality, one belonged to the same subclade as the sequence of case 1, whereas the remaining eleven shrew-derived sequences were more closely related to the sequence of case 2 (Fig. 3). Based on the partial genomic sequences used for the phylogenetic analysis (1824 nt, covering the N, X and P genes), the highest pairwise nt sequence identities for the two human cases were 99.8 % for case 1 (to shrew KS22/2365, GenBank OR450010) and 100 % for case 2 (to shrew KS23/0313; OR450013).

4. Discussion

Applying the holistic One Health approach, we conducted an in-depth investigation of the first detected local cluster of two fatal human BoDV-1-infections. We gained insights into the seroprevalence of BoDV-1 in a adult population with risk for BoDV-1 exposure, and we further obtained knowledge on the local shrew community and the presence of BoDV-1 within the reservoir population.

4.1. Sero-epidemiological investigation (BOSPEK-study)

Before the zoonotic potential of BoDV-1 was proven and linked to severe human cases of encephalitis in 2018, controversial debates in the scientific community on the seroprevalence of BoDV-1 and a possible association with psychiatric disorders had occurred (reviewed in [6,7]). Acknowledging the zoonotic aspect of BoDV-1 infections, the aim of the BOSPEK-study was to systematically assess the BoDV-1 seroprevalence in a defined adult population of a municipality with documented recent human infections in an endemic region. This unique study design differentiates from the approach used in earlier studies assessing the prevalence of anti-BoDV-1 IgG antibodies, in which the spatial origin of the tested individuals was less clearly defined. In a previous study, 1109 participants (healthy blood donors and veterinarians) were included [28]. One serum sample of a veterinarian was tested positive by IFAT. According to the questionnaire, the person suffered from joint pain, but the further fate of the person could not be followed due to the anonymized study design [28]. Another study tested samples of 216 healthy blood donors and samples of 280 outpatients after solid organ transplantation, as well as 288 serum and 258 CSF samples of patients with encephalitis and a request for tick-borne encephalitis virus (TBEV) diagnostics [24]. A positive serological test result, accompanied by the detection of BoDV-1-RNA in the corresponding CSF sample and brain samples subsequently collected during autopsy, was obtained from one patient who suffered from a previously unspecified severe neurological disease [24]. Two nation-wide studies [8,10] showed the presence of bornavirus-reactive antibodies or BoDV-1-RNA only in patients with encephalitis in virus-endemic regions and neither in asymptomatic individuals, nor in a total of 556 patients with various other neurological or psychiatric conditions from across Germany. In line, also serological screening of household members of patients with confirmed BoDV-1-encephalitis was negative according to another study [20].

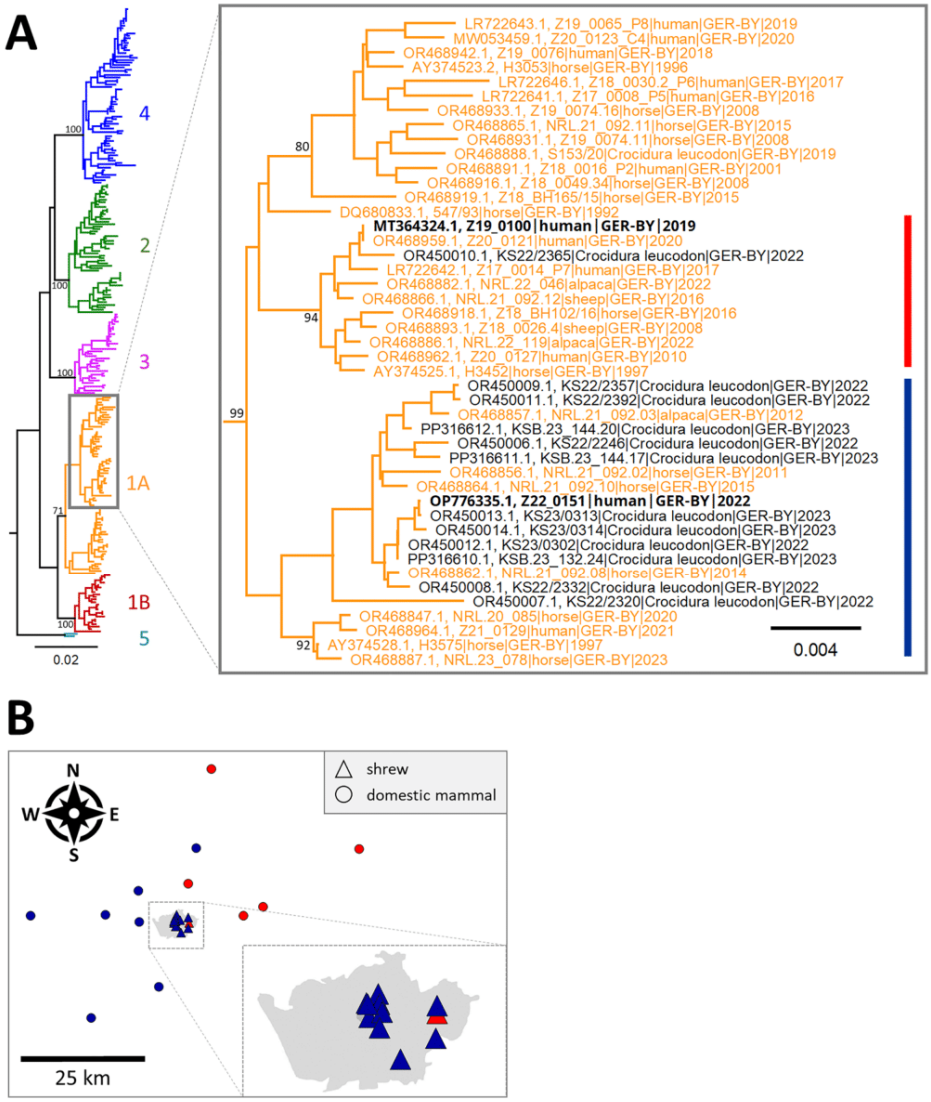

Fig. 3. Phylogenetic analysis of BoDV-1 sequences of human and animal origin. (A) A Jukes-Cantor neighbour-joining tree was calculated for partial BoDV-1 sequences (1824 nt, covering the N, X and P genes of the BoDV-1 genome) from shrews and human cases from the municipality (depicted in black) together with sequences from shrews, domestic mammals and humans from the endemic regions in Germany, Austria, Switzerland and the Principality of Liechtenstein (total 258 BoDV-1 sequences) using Geneious Prime 2021.0.1. The tree was rooted with sequence BoDV-2 No/98 (AJ311524, not shown). Values at branches represent support in 1000 bootstrap replicates. Colors of the branches represent previously published BoDV-1 clusters 1A, 1B and 2 to 5 [2,4,7–9,11,20,27,30]. Horizontal bars (red, blue) indicate the phylogenetic subclades present in the municipality. Only bootstrap values ≥70 at major branches are shown. GER: Germany; BY: Bavaria. **(B)** Spatial mapping of BoDV-1 sequences from shrews and domestic mammals relative to the location of the municipality (grey silhouette). Colours represent the phylogenetic subclades of cluster 1A present in the municipality. Human cases are not presented due to data protection policy. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the BOSPEK study, no IFAT-confirmed BoDV-1-specific IgG antibodies were detected in serum samples, whereas the frequency of ELISA reactivities regarded as non-specific were comparable to previous studies [24]. Autoantibodies against expressed endogenous bornavirus-like (EBL) elements in the human genome or cross-reactive antibodies against other infectious agents (e.g. bacteria) were hypothesised as potential causes of unspecific reactivities in this serological test based on *E. coli*-derived recombinant BoDV-1 proteins [24]. For the testing of T cell responses examined in a subgroup of our BOSPEK-study, two PBMC samples exhibited a borderline BoDV-1-ELISpot result, but together with the negative serological findings was interpreted as unspecific. In conclusion, BoDV-1-infections appear to be rare even in endemic regions and seem to primarily result in severe neurological disease with a high case-fatality rate.

4.2. Epidemiological investigation

The epidemiological part of the BOSPEK-study revealed frequent presence of shrews in the participants' living environment, and every fifth participant even reported direct shrew contact and/or contact to the excreta of mice and/or shrews. Accordingly, at least a potential risk of exposure to BoDV-1 for individuals living in the municipality can be assumed. However, infection with subsequent development of BoDV-1-related encephalitis occurs only extremely rarely – even in endemic areas such as the municipality at centre of this investigation. This may indicate the presence of previously unknown cofactors. Nevertheless, the population should be sensitized to the risk of BoDV-1 in affected regions.

4.3. Tenacity of BoDV-1 in the environment

Our tests on BoDV-1-tenacity show that the virus can remain infectious for several days at room temperature under dry and humid conditions. These data extend previous findings showing that BoDV-1 can survive for up to two weeks at ambient temperatures in sterile fluids [19]. Specifically, our experiments mimicked natural conditions by pouring BoDV-1-containing fluids onto leaves and herbs. However, these fluids were derived from cell culture supernatants, and therefore we cannot exclude that BoDV-1 behaves differently in the urine or faeces of infected shrews.

4.4. Environmental investigation

The isolation of a mammalian orthoreovirus from a small mammal burrow further confirms that the selected conditions appear to be suitable for the analysis of environmental samples, although it remains unclear whether BoDV-1 can be isolated from samples collected in the field using this method. We were unable to detect BoDV-1-RNA or infectious virus in any of the environmental samples collected, which is in line with previous limited sampling attempts for BoDV-1-RNA in soil [18]. One reason may be that the overall sensitivity of the applied methods may be too low to detect small traces of BoDV-1 in the environment or that we collected in places not frequented by shrews. Since knowledge regarding the behaviour and habitats of *C. leucodon* are scarce, it is a priority to evaluate the presence of *C. leucodon* populations, especially in places with BoDV-1-infections. This would allow improved environmental studies and more targeted recommendations for the public. Another possible explanation may be the collection of the environmental samples on a sunny day with temperatures above 32 °C (90 °F). We took great care to sample in shaded areas, but heat and UV radiation may have had detrimental effects on BoDV-1 RNA and infectivity.

4.5. Tick investigation

Neither evidence nor indications for tick-borne BoDV-1-transmission have been reported so far, which is in line with the lack of tick bite history of BoDV-1-infected patients prior to the onset of disease [18]. Nevertheless, the possibility of transmission via arthropod-vectors (ticks and other) deserves further investigation. Our study is the first to test for BoDV-1 in ticks in direct association with human infections. However, BoDV-1-RNA could not be detected in any of the 336 specimens.

4.6. Small mammal investigations

This is the first comprehensive study of the small mammal community in the context of confirmed human BoDV-1-infections in an endemic area. Due to substantial public awareness and support, we were able to establish a very fruitful citizen science project. The investigations confirmed the presence of BoDV-1-RNA in the local *C. leucodon* population with a positivity rate of 30 % (12 out of 40). 39 of these shrews were found dead and were contributed by inhabitants of the municipality, whereas only one was caught by active trapping.

The numbers may have been influenced by our non-systematic sampling and by hunting habits of cats, as positive shrews were cat preys or found dead. Due to this fact, the precise location of the positive individual habitats could not be determined, as the location of hunting may differ from the place where the carcass was found. However, the owners of the cats that provided BoDV-1-positive shrews lived in different parts of the village (**Figure S10**), suggesting a broad distribution of the virus within the region.

To address this limitation, active snap trapping was performed. The sites were selected based on recommendations by the citizens and of public interest (soccer field/tennis court, playground, gardens of the deceased), rather than ideal *C. leucodon* habitats, which may have led to low trapping success of only three shrews (*Sorex araneus, Neomys anomalus, C. leucodon*, one each). In general, shrews are more difficult to trap than rodents, making citizen science projects of even greater value for pathogen studies in shrews. The single *C. leucodon* was trapped in a stack of green-cuttings, which is typically used for nesting. We can confirm their presence at the one site (soccer field/tennis court), which represents a high-contact zone between many people and shrews, and was also regularly frequented by both human cases. Of note, this particular shrew tested negative for BoDV-1, but this does not exclude the viral presence at that specific site, as only one third of all investigated *C. leucodon* were positive for BoDV-1. Other studies, which investigated small mammals from regions with BoDV-1-infected animals and/or humans reported similar detection rates of 10 % to 67 %, albeit the number of investigated *C. leucodon* and timeframes varied [4,5,29,30].

The extent of the human-shrew interface is most likely the main risk factor for BoDV-1-infections, which increases with rural living conditions [18]. Even though citizens have described to have seen shrews nearby or even had direct contact, our study on the small mammal community composition demonstrated that only a minority of the shrews were *C. leucodon* (18 %; 40/220). However, with a detection rate of 30 %, the overall detection rate of BoDV-1-RNA-positive *C. leucodon* amongst all investigated shrews is only 5.4 % Therefore, direct shrew contact is not necessarily equivalent to the encounter of a BoDV-1-positive shrew. Even so, direct and indirect contact to these elusive animals should be reduced as much as possible. Although the proportion of people unable to correctly identifying a shrew was as low as 7 % in our study, citizens of BoDV-1-endemic areas should be trained to recognise shrews or rather be able to differentiate between mice and shrews. Recommendations on personal protective equipment, when performing higher-risk activities, such as discarding carcasses as well as recommendations to reduce unnatural feed resources (e.g. cat/dog food, food scratches), should be provided to the public. Whether or not cat owners are at greater risk than non-cat owners, due to cats carrying BoDV-1-infected shrews into human dwellings, remains questionable and cannot be determined yet based on the small number of reported human cases [8,18]. The relatively large number of collected *Neomys* spp. was unexpected, but could be due to sampling bias as *Neomys* spp. and *C. leucodon* have a similar appearance and citizen being more alert to collect crocidurine-like shrews (**Figure S11**).

4.7. Phylogenetic investigation

The BoDV-1 sequences of human cases 1 and 2 belonged to different phylogenetic subclades within BoDV-1 cluster 1A, suggesting independent infection sources. Within this study, we identified shrew-derived BoDV-1-sequences from either subclade, with complete or almost complete identity to either of the human cases. This confirms the likely peridomestic infection sources for both patients. The analysis of BoDV-1 sequences from human and animal patients from surrounding districts indicates that the dispersal areas of both subclades overlap within the area of the municipality.

4.8. Limitations

The studies conducted within this One Health project are subject to some limitations that should be acknowledged. Previous studies have shown that many patients suffering from confirmed severe BoDV-1 encephalitis had still been seronegative at the time of hospitalization and BoDV-1-reactive antibodies became detectable only relatively late in the course of the disease [8,10,20]. Based on the study approach and the presently available laboratory tests, it cannot be excluded that early stages of BoDV-1 infections may have been missed. However, as of February 2024 no further BoDV-1-cases have been reported from the municipality.

Moreover, environmental sampling was hampered by the fact that

we did not know which sites were regularly frequented by shrews. Even though our established citizen science project within the framework of the reservoir investigation was of great success delivering the majority of investigated shrews, a conclusion about the exact origin of BoDV-1-positive bicoloured white-toothed shrews could not be drawn and hence the determination of putative infection sites and routes was not possible.

4.9. Conclusions

Although more than 50 human BoDV-1-encephalitis cases were reported to public health authorities so far, little is known about the transmission setting, infection routes to humans, and risk factors. In our study, BoDV-1 was detected in the known reservoir shrew population in the municipality and residents reported regular direct or indirect shrew contact. However, we found no evidence of BoDV-1-specific seroconversion and, thus, also no serological indications of asymptomatic or clinically atypical human infections other than the known severe encephalitis cases reported. We found no trace of the virus in either the environment or in ticks, underscoring the difficulty in uncovering transmission routes to humans. The phylogenetic analysis of the BoDV-1-sequences from the two patients highly suggests a local infection for both patients but possibly at different sites, as viral sequences belong to two different subclades. Both sequence types were also found in the local *C. leucodon* populations. The proportion of BoDV-1-positive *C. leucodon* (30 %) is in congruence with the results of previous studies from other regions and proves the presence of BoDV-1 in the municipality. Of note, the proportion of BoDV-1-positive *C. leucodon*amongst all tested shrews was low (5.4 %). Direct or indirect contact with infected shrews is therefore likely to be limited and might explain the sparsity of human BoDV-1-infection despite viral presence in the natural reservoir species. Moreover, further research is needed to narrow down transmission routes. Additionally, targeted information campaigns as well as ecologically sound measures maintaining biodiversity are needed to limit the human-shrew interface in order to prevent human infections in endemic areas.

Ethics

The BOSPEK-study was approved by the Ethics Committee of the University of Regensburg, Germany (reference number 22-2976-101) and conducted in accordance with the Declaration of Helsinki after registration in the German Clinical Trials Register under the reference number DRKS00029412 (registered on 01/07/2022). All participants (n −679) were included after written informed consent. For the trapping of small mammals in the affected municipality a trapping permit from the Government of Upper Bavaria was available (number: ROB-55.1-8646. NAT_03-4-76-13).

CRediT authorship contribution statement

Merle M. Böhmer: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Viola C. Haring:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation. **Barbara Schmidt:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Franziska S. Saller:** Writing – review & editing, Formal analysis, Data curation. **Liza Coyer:** Writing – review & editing, Formal analysis, Data curation. **Lidia Chitimia-Dobler:** Writing – review & editing, Resources, Investigation. **Gerhard Dobler:** Writing – review & editing, Resources, Investigation. **Dennis Tappe:** Writing – review & editing, Funding acquisition. **Andrea Bonakdar:** Writing – review & editing, Investigation. **Arnt Ebinger:** Writing – review & editing, Investigation. **Gertrud Knoll:** Writing – review & editing, Investigation. **Lisa Eidenschink:** Writing – review & editing, Investigation. **Anette Rohrhofer:** Writing – review & editing, Investigation. **Hans Helmut Niller:** Writing – review & editing, Resources, Investigation. **Katharina Katz:** Writing – review & editing, Conceptualization. **Philip Starcky:** Writing – review & editing, Investigation. **Martin Beer:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rainer G. Ulrich:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Dennis Rubbenstroth:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Markus Bauswein:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2024.105658.

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V. Discussion

Detection of the zoonotic Langya virus (genus *Henipavirus*) in white-toothed shrews, namely *Crocidura lasiura* and *Crocidura shantungensis*, in China and the identification of *Crocidura leucodon* as reservoir of the lethal zoonotic BoDV-1 have drawn white-toothed shrews into the attention only lately.

The successful involvement of the public to monitor wildlife for conservation and ecological purposes has been demonstrated (Frigerio et al., 2018; Weinberger and Briner 2021; Silvertown et al., 2013). We have established a very fruitful citizen science project, where the public sent in shrews found dead for unknown reason or preyed by cats. This enabled us to study the ecology and pathogen prevalence of the very elusive and difficult to monitor white-toothed shrews. These species are protected by the federal nature conservation act (BNatSchG, revised 12/8/2022; Meinig et al., 2020; BArtSchV, revised 1/21/2013) and not considered pest animals. The citizen science project was supported by bycatches from ongoing small mammal monitoring projects of various collaborators of the previously established network “Rodent-borne pathogens” (Ulrich et al., 2009).

A variety of important issues were raised trying to determine the role of white-toothed shrews in Central Europe as putative reservoirs of known and as-yet-unknown zoonotic and enzootic pathogens, three of which are further explored in this thesis.

First, the distribution of white-toothed shrews and composition of the shrew community investigated in a highly endemic BoDV-1 area to identify potential interfaces for spillover infections;

second, a screening approach was performed for both known and as-yet unknown pathogens using pathogen-specific and generic PCRs, and an unbiased mHTS approach to determine pathogen diversity; and **third**, a holistic One Health study investigating the role of the environment, the animal reservoir and the situation within the human population, was conducted to assess all aspects of human BoDV-1 infection in the municipality with the first described disease cluster.

Contribution to the description of the current distribution of white-toothed shrews in Germany and determination of the shrew community in a high endemic area of BoDV-1.

Publication 1, Publication 3

The current distribution of white-toothed shrews in Germany is only incompletely described and frequently based on the evaluation of skeletal remains found in barn owl pellets (*Tyto alba*) (Weise 2011; Schmitt 2017; Biedma et al., 2019).

Despite our semi-structured sampling strategy, which relied heavily on the compliance of the public and on bycatches from small mammal monitoring projects, the previously described distribution ranges could be confirmed. Species identification of shrews was based on molecular analysis of the *cytochrome b* gene, which enables in accurately identifying the species and provides valuable information on the present species composition. These data enable further phylogenetic analysis to evaluate the dynamics and structures of populations, solely or in conjunction with pathogen evolution and disease dynamics (Dubey et al., 2007a; Dubey et al., 2008b; Drexler et al., 2015).

The distribution ranges of the three white-toothed shrews present in Western and Central Europe overlap in Germany (Wilson and Mittermaier 2017). Dynamics of the distribution ranges of the *Crocidura* species have been reported in the literature, such as an ongoing eastward expansion of *Crocidura russula* (Frank 1983; Borkenhagen 1995; Kraft et al., 2010; van der Kooij and Nyfors 2023; Bellocq et al., 2023) and westwards expansion of *C. suaveolens* (Jentzsch and Trost 2008) and *C. leucodon* further north (Klesser et al., 2021). Of note, the latter shrew species are outcompeted by the larger *C. russula* leading to their local extinction, which has been demonstrated for *C. leucodon*, *C. suaveolens*, and *S. coronatus* (Kraft 2000; Vogel et al., 2002; McDevitt et al., 2014). During the past years, an overall reduction of insectivore species in the diet of barn owls has been observed. However, the population of the synanthropic *Crocidura* remained stable compared to other shrew species (Roulin 2016). This is in contrast with older descriptions that have observed a decline in *C. leucodon* and *C. russula* (Lehmann and Brücher 1997), demonstrating fluctuation of the population density and distribution. *Crocidura russula* predominantly occurred in the western half of Germany and has only been detected sympatrically with *C. leucodon* at five sites in central Germany. *Crocidura leucodon* and *C. suaveolens* were mainly present in the eastern half of Germany (Schmidt 1998, 2019), and occurred in the study here sympatrically at four sites. *Crocidura leucodon* had overlapping distribution ranges with the other two species (**Publication 1**). Concentrated studies of the small mammal community composition at a small municipality in Bavaria, Germany, where the first human BoDV-1 infection cluster was described, demonstrated a low abundance of *C. leucodon*, and absence of *C. russula* and *C. suaveolens*, which is in accordance to the expected species distribution in this region (Kraft 2008) (**Publication 3**).

Next to identifying the distribution of the shrew species, pathogen diversity present in the animal host needs to be evaluated to determine potential infection risks. Within this thesis, this has been accomplished by applying generic and pathogen-specific PCRs on a large sample set of almost 400 bicolored, greater and lesser white-toothed shrews from multiple European countries, and by an unbiased mHTS approach. The inclusion of *Suncus etruscus* in the sample subset of the latter study is justified considering its presence in captivity, despite its absence in the wild in Germany and the majority of Central Europe (Wilson and Mittermaier 2017; Geyer et al., 2022; Zootierliste 2023; Vogel 2012). The results obtained from the mHTS approach will be addressed later.

Determining the presence of selected pathogens in white-toothed shrews from various European countries.

Publication 1, Publication 2

Pathogen diversity in white-toothed shrews worldwide was demonstrated, but knowledge on crocidurine species from Central Europe are lacking. (**Table 2** and **Table 3**). Compared to the limited knowledge on crocidurine shrews, rodents and soricine shrews have been more extensively surveyed in temperate regions (Bray et al., 2007; Bown et al., 2011; Radosa et al., 2013; Mayer-Scholl et al., 2014; Obiegala et al., 2019).

Small mammals, particularly rodents, play an essential role in hosting arthropods such as ticks and fleas, and hence act as vector for arthropod-borne pathogens (Mihalca and Sándor 2013; Krawczyk et al., 2020). *Neoehrlichia mikurensis* is a tick-borne pathogen associated with a systemic inflammatory syndrome in immunocompromised human patients described in Germany, Switzerland, the Czech Republic, Sweden, and China (Li et al., 2013; Andersson and Råberg 2011; Silaghi et al., 2016). It is transmitted by hard ticks (*Ixodes* spp.) such as castor bean ticks (*Ixodes ricinus*), and was previously mainly detected in rodents (Andersson and Råberg 2011; Silaghi et al., 2012; Jahfari et al., 2012). It was not detected in the West European hedgehog samples (*Erinaceus europaeus*) from our study, although low prevalence in Northern white-breasted hedgehogs (*Erinaceus roumanicus;* 2.3%*)* from Hungary has been reported (Földvári et al., 2014). It was assumed that shrews play no role in the infection cycle and transmission (Obiegala et al., 2014; Silaghi et al., 2016). However, our findings demonstrate the susceptibility of at least *C. russula* for *N. mikurensis* as we detected two *N. mikurensis*-DNA positive *C. russula* from distant trapping sites in North Rhine-Westphalia and Thuringia (**Table 4**). Further research is required to determine whether these were spillover from sympatric rodents, hence rare incidents, or if shrews pose an infection risk for *N. mikurensis* infection in humans. *Bartonella* spp. and *Anaplasma* spp. were previously detected in white-toothed shrews outside of Germany (*C. suaveolens* in Turkey (Celebi et al., 2021), *C. russula* in France and Spain (Krügel et al., 2022)). A unique *Bartonella* strain, *Bartonella florenciae*, was isolated from *C. russula* in France (Mediannikov et al., 2013) (**Table 3**). Babesiosis caused by different *Babesia* spp. is an emerging disease worldwide (Hunfeld and Brade 2004), causing high economic losses in livestock production (WOAH 2021), and may cause life-threatening infections in humans. *Babesia microti* is associated with the vast majority of North American human babesiosis cases and autochthonous cases have been described in Europe (Hildebrandt et al., 2021). It was reported from multiple small mammal species (Obiegala et al., 2015; Hamšíková et al., 2016). Bown et al., (2011) reported an equally high prevalence of *B. microti* in *S. araneus* as in field voles (*Microtus agrestis*) from the same habitat. Our investigations resulted in no detection of *Bartonella* spp., *A. phagocytophilum* and *Babesia* spp. in the white-toothed shrew

samples, but yielded positive results for *E. europaeus*, demonstrating the circulation of *Bartonella* spp. and *A. phagocytophilum* in Germany. The ectoparasite milieu specific to a species has been evaluated for other reservoir hosts, but not yet for white-toothed shrews specifically (Ko et al., 2011; Obiegala et al., 2021; Colombo et al., 2023). The overall low prevalence of arthropod-borne pathogens among white-toothed shrews of this study might be attributable to a species' specific ectoparasite milieu and/or to specific ecological and behavioural features of the animals, and thus warrants further investigation.

Table 4 Summary of the pathogens detected in white-toothed shrews of this thesis (**Publication 1-3**). The total number of investigated individuals is a summary of white-toothed shrews across all three publications, which were investigated for the presence of the respective pathogen.

Pathogen		Method	**Greater white-toothed shrew** (*C. russula*) positive individuals/ total number investigated	**Bicolored white-toothed shrew** (*C. leucodon*) positive individuals/ total number investigated	**Lesser white-toothed shrew** (*C. suaveolens*) positive individuals/ total number investigated	**Etruscan shrew** (*S. etruscus*) positive individuals/ total number investigated
Leptospira spp.	*Leptospira kirschneri* (ST 100)	qPCR, SLST, MLST	**28**/227	**3**/81*	0/22	-
	Brucella spp.	qPCR	0/213	0/80	0/21	-
	Coxiella burnetii	qPCR	0/213	0/80	0/21	-
Arthropod-borne	*Neoehrlichia mikurensis*	qPCR	**2**/213	0/80	0/21	-
	Bartonella spp.	PCR	0/213	0/80	0/21	-
	Anaplasma phagocytophilum	qPCR	0/213	0/80	0/21	-
	Babesia spp.	PCR	0/213	0/80	0/21	-
Paramyxoviridae	Denwin virus	mHTS + RT-qPCR	**9**/16	0/21	0/6	0/2
	Hasua virus	mHTS + RT-qPCR	0/16	0/21	**1**/6	0/2
	Resua virus	mHTS + RT-qPCR	0/16	0/21	**3**/6	0/2
	Lechcodon virus	mHTS + RT-qPCR	0/16	**2**/21	0/6	0/2
Nairoviridae	Erve virus	mHTS + RT-qPCR	**2**/16	0/21	0/6	0/2
	Regana virus	mHTS + RT-qPCR	0/16	**7**/21	0/6	0/2
	Rasenna virus	mHTS + RT-qPCR	0/16	0/21	0/6	**2**/2
Hepeviridae	*Paslahepevirus crocidurae*	mHTS + RT-qPCR	**2**/16	0/21	0/6	0/2
Bornaviridae	Borna disease virus 1	RT-qPCR	**1**/16	**14**/42	**2**/6	0/2

-: species not investigated; *: including *Leptospira* spp. DNA positive *C. leucodon* from Jeske et al., 2021

Coxiella burnetii and *Brucella* spp. are zoonotic bacteria causing abortions in livestock and can be transmitted to humans through the consumption of unpasteurized dairy products or by close contact with excretions of carrier animals. Risk groups are veterinarians, farmers, and abattoir workers. These pathogens can cause febrile diseases such as Q-fever (*C. burnetii*) and multi-organ failure in humans (brucellosis). Socio-economic impacts such as financial losses and e.g. their consequential effects on farmers' livelihoods, need to be considered to an equal extent as their direct health threat, when estimating the disease burden (Lopez et al., 2012; Smith et al., 2019). This was further emphasized by

the One Health agenda (Adisasmito et al., 2022; Dean et al., 2012). Eradication programs for brucellosis in cattle and small ruminants were successfully established and Germany is considered to be free of caprine/ovine and bovine brucellosis since 2000 (FLI 2022). Wild animals are known reservoirs for different *Brucella* species (Moore and Schnurrenberger 1981; Godfroid et al., 2013), or may tend to establish natural foci after spillover infections from domestic animals (Zheludkov and Tsirelson 2010). Thus, monitoring of wildlife, such as ubiquitous small mammals, is necessary to safeguard the brucellosis-free status of farms from reintroduction of *Brucella* spp. through wildlife (Scholz et al., 2009; Scholz et al., 2008; Rónai et al., 2015). Previous studies found 8% of soricine shrews positive for *Brucella* spp. (Hammerl et al., 2017). However, we could not detect any *Brucella* spp. and *Coxiella burnetii*-DNA in the specimens studied (**Table 4**), indicating an extremely low, if any, incidence. This implies variations in pathogen diversity in crocidurine and soricine shrews but may also be biased by the trapping site.

The *Leptospira* prevalence of 12.3% in *C. russula* and 3.7% in *C. leucodon* of this study is lower, but still within the range of mean *Leptospira* prevalence of soricine shrews in Germany described by Fischer et al., (2018) (3.0% - 15.5% for *S. coronatus* and *S. araneus*, respectively). This is not unexpected as soricine shrews tend to thrive in cooler and wetter environments, whereas crocidurine shrews predominantly inhabit open and dry areas. Interestingly, Kraft and colleagues have caught *C. leucodon* along small river stretches in a forest area (Kraft et al., 2010), which was previously not considered as suitable habitat for *C. leucodon*. This may indicate alterations in the habitat preferences of *C. leucodon* possibly influencing future pathogen prevalence.

Further characterization of the *Leptospira* species by SLST targeting the *secY* gene identified pathogenic *Leptospira kirschneri*. The identification of the ST by MLST was possible for *C. russula* samples demonstrating the exclusive presence of *Leptospira kirschneri* ST 100 in all samples. This contrasts with previous studies where *Leptospira kirschneri* ST 110 and ST 136 were detected in *S. araneus* and *L. borgpetersenii* ST 146 in *S. araneus* and exclusively in *S. coronatus.* The detection of only one ST in the investigated crocidurine shrews may indicate an association between this ST and white-toothed shrews, although it has previously been reported from a Portuguese house mouse (*Mus musculus*) (Ferreira et al., 2020) and was also detected in *E. europaeus* in this study (**Publication 1**).

In summary, white-toothed shrews appear to have a minor role in the transmission of bacterial pathogens compared to rodents. However, there are knowledge gaps in relation to the investigated pathogens (*N. mikurensis)*, which could be addressed to further advance research in this area.

Identification of the virome of white-toothed shrews with a metagenomic high-throughput sequencing approach.

Publication 2, Review 1

Advances in sequencing have led to the implementation of unbiased mHTS, which has proven a useful tool to study viral diversity in wildlife species (He et al., 2013; Smith and Wang 2013), altering our understanding of the global virosphere (Zhang et al., 2019). Detailed information on the virus distribution and molecular evolution aid to track cross-species events with pre-pandemic potential (Koonin and Dolja 2018; Zhang et al., 2018). Host-microbe synergism was highlighted by the amount of pathogens harmlessly residing in invertebrates (Zhang et al., 2019) and an upscale of the diagnostic speed of rare and previously unknown pathogens could be observed (Wilson et al., 2019; Belák et al., 2013). However, these studies are often restricted by limitations in data analysis, data standardization, and by a lack of further down-stream characterization (Letko et al., 2020; Cobbin et al., 2021).

Here we present a blueprint for metagenome studies in neglected putative reservoir species. For enhanced sequencing success relative abundance of viral RNA was increased by host-rRNA (ribosomal RNA) depletion (siTOOLs Biotech). Trimmed contigs were compared to existing databases. A moderate to high viral abundance of viruses of the orders *Mononegavirales, Picornavirales, Stellavirales, Hepelivirales,* and *Bunyavirales* was detected across all white-toothed shrew samples. Whole genomes were generated, and phylogenetic analyses demonstrated a close relationship to highly virulent pathogens of global importance. Therefore, we could illuminate the viral diversity in white-toothed shews in Central Europe, which contrasts with the previously low detection of bacterial pathogens within the same sample set (**Publication 1**).

Virus-specific RT-qPCRs were designed to investigate viral RNA tissue tropism and ascertain the principal target organs along with potential excretion and transmission routes. Low cycle threshold values (ct-values) and relatively broad viral RNA tissue distribution were demonstrated for all novel viruses pointing towards their persistence within the organism and their potential for further shedding via various routes. Confirmation of viral presence in the original samples by RT-qPCRs is important to distinguish between contamination during sequencing (Asplund et al., 2019; Holmes 2019) and actual presence in the respective individual (Cobbin et al., 2021)

Of special interest were members of the families *Nairoviridae*, *Paramyxoviridae*, *Hepeviridae* and *Bornaviridae*. Important representatives of *Paramyxoviridae* with host-pathogenicity are CDV in dogs, Newcastle disease (NDV) in birds, Sendai virus (SeV) for rodents, and Rinderpest virus (RPV) for livestock (Rima et al., 2019). A large-scale investigation in bats and rodents by Drexler et al., (2012) had revealed 66 novel paramyxoviruses of five subfamilies. Rodents from Germany harboured only

novel strains of the genus *Jeilongvirus,* which is a primarily rodent-borne paramyxovirus group (Ghawar et al., 2017; Mohd-Qawiem et al., 2022).

Whole genomes of four distinct paramyxoviruses were detected in all investigated shrew species besides *S. etruscus*: novel Hasua virus (HasV) and Resua virus (ResV) in *C. suaveolens,* Lechcodon virus (LechV) in *C. leucodon*, and the previously described Denwin virus (DewV) in *C. russula* (Vanmechelen et al., 2022). Those shrew-derived sequences are distant from bat-borne and rodent-borne paramyxoviruses of other genera and form their own shrew-derived sister clade within the genus *Henipavirus*. Interestingly, HasV is next of kin to LayV, which was recently isolated from hospitalized febrile patients (Zhang et al., 2022b). All 35 human LayV-cases were independent from each other but had in common to have a lived rurally with a potential (small) mammal exposure. Screening of 25 small mammal species (n=3380) revealed that LayV-RNA was mainly present in *C. lasiura* (52%) and *C. shantungensis* (20%) (Zhang et al., 2022b), and only to minor degrees in sympatric rodents. This may indicate a moderate to low host-specificity typical for members of the genus *Henipavirus.*

Paramyxoviruses are most likely more diverse and globally distributed (Drexler et al., 2012; Vanmechelen et al., 2022) than previously assumed, as evidenced by the ongoing discovery of novel paramyxoviruses in shrews around the world, i.e. in Africa (Sasaki et al., 2014; Kleinhans 2022; Vanmechelen et al., 2022), and Asia (Chen et al., 2020a; Lee et al., 2021) (**Table 2**).

Interestingly, paramyxoviruses distant to LayV were detected in *C. shantungensis* and *C. lasiura* from the Republic of Korea (Lee et al., 2021). *Crocidura shantungensis* was formerly considered a member of the *C. suaveolens sf.* species complex, which extends from Europe towards China (Wilson and Mittermaier 2017; Gritsyshin et al., 2023), and was only recently clearly identified as separate species (Bannikova et al., 2009; Lee et al., 2018; Chen et al., 2020b). The global distribution of paramyxoviruses in shrews especially in those closely related, may suggests an ancient presence of paramyxoviruses and a certain degree of host-pathogen co-evolution with shrews.

Prominent representatives of the genus *Henipavirus* are HeV and NiV, which were first described in the late 1990s in Australia and South-East Asia, respectively (Murray et al., 1995; Chua et al., 2000). Nipah virus is detected in recurring outbreaks primarily in Bangladesh and India (Islam et al., 2023b), with an ongoing outbreak as of September 2023 in the Indian state Kerala, its fourth outbreak since 2018 (Crawford 2023). Both viruses are of considerable animal and public health concern, due to their high morbidity and mortality, symptoms ranging from severe respiratory distress to encephalitis. Mortality rates in humans range between 40-70% (WHO 2018). The absence of approved antiviral postexposure treatment and effective human vaccines led to their classification as Biosafety level 4 (BSL 4) pathogens (Gómez Román et al., 2022). The World Health Organization (WHO) has developed a roadmap for the treatment and vaccine development for top priority pathogens such as HeV and NiV (WHO 2019). Equivac® HeV is the first commercially available vaccine against HeV licensed for

immunization of horses in Australia (CSIRO 2021; Wang et al., 2023b), and vaccine candidates are developed for immunization of pigs against NiV infections (Weingartl et al., 2006). So far, information on pathogenicity, virulence, transmission and thereby zoonotic potential is lacking for the novel shrew-derived paramyxoviruses. Due to their phylogenetic relatedness, one may expect properties similar to LayV, HeV and NiV. The shrew-borne paramyxoviruses may represent interesting substitute candidates for the development of post-exposure treatment and human vaccines against HeV and NiV (Delwart 2012).

The transmission from fruit bats as natural reservoirs to human dead-end hosts, requires horses and pigs (HeV, Niv respectively) as intermediate hosts, which also exhibit clinical signs (Chua et al. 2002). However, bat-to-human transmission and rare human-to-human transmission have been described for NiV (Gurley et al., 2017; WHO 2019, 2018; Gazal et al., 2022). If direct transmission of LayV and related viruses from shrews is possible, or if an intermediate host is required, needs to be further elucidated. Tissue distribution demonstrated low ct-values in kidney tissue, suggesting virus excretion via urine facilitating rapid horizontal transmission. Possible scenarios for virus exposure include urine marking for territorial defence (Wilson and Mittermaier 2017; Geyer et al., 2022), which could serve as an infection route through contaminated food. This was demonstrated for raw date palm sap contaminated with bat-derived body fluids containing infectious NiV (Rahman et al., 2012; Gurley et al., 2017; Gazal et al., 2022). Identification of an appropriate target organ will guide the success of large-scale screenings (Drexler et al., 2012).

Well-known representatives of the family *Nairoviridae* are those closely related to the Crimean-Congo haemorrhagic fever virus (CCHFV*),* such as Hazara virus (HAZV), Dugbe virus (DUGV) and Nairobi sheep disease virus (NSDV), which can cause severe disease in humans and/or (domestic) animals, respectively. In recent years, nairoviruses have been identified in multiple (mammalian) species, demonstrating a global distribution (Honig et al., 2004). Within this study distinct nairoviruses were identified in three of the four investigated shrew species. Whole genomes were generated for Rasenna virus (RASV) in *S. etruscus* and for Regana virus (REGV) in *C. leucodon* with high interspecies sequence variability. Erve virus (ERVEV*)* was identified as potential causative agent of thunderclap headache in humans (Treib et al., 1998) and was isolated from the spleen of *C. russula* from France (Chastel et al., 1989; Dilcher et al., 2012). Here we present whole genomes of ERVEV in *C. russula* from Germany and demonstrate the ongoing presence of this virus in Europe. So far, only serological investigations detecting anti-ERVEV-antibodies have been conducted to estimate the presence of ERVEV in both animals and humans (Treib et al., 1998; Woessner et al., 2000). The new sequences will assist in developing molecular diagnostic techniques such as (real-time) RT-PCR, which is helpful to assess the zoonotic potential of ERVEV, and to overcome the limitations of antibody cross-reactivity and non-specificity associated with serological assays. The presence of related, yet unknown, viruses

in wildlife species must be considered when developing diagnostic tests, as close antigenic relationship may lead to cross-reactivity affecting the outcome of test results.

Phylogenetic analysis of the newly described nairoviruses demonstrated the close phylogenetic relationship to ERVEV and Thiafora virus (TFAV), which is a shrew-borne nairovirus detected in *Crocidura* spp. from Senegal (Zeller et al., 1989). Additional members of the Thiafora group were detected in shrews outside Europe such as Cencurut virus (CENV) in *S. murinus* in Singapore (Low et al., 2023), and Lamusara (LMSV) and Lamgora (LMGV) virus detected in the Goliath shrew (*Crocidura goliath*) from Gabon (Ozeki et al., 2022) (**Table 2**). Next to ERVEV, the description of nairoviruses present in small mammals in Central Europe was recently extended by the identification of the zoonotic Issy-kul virus (ISKV) in bats (Brinkmann et al., 2020; Cholleti et al., 2022). Transmission of CCHFV and relatives is dependent on ticks, especially *Hyalomma* species (Estrada-Peña et al., 2012; EFSA-AHAW 2013; Gargili et al., 2017). Reports of the first autochthonous CCHFV-infection in humans in Spain (Negredo et al., 2019; Negredo et al., 2017) indicate the potential establishment of enzootic cycles of this lethal virus and relatives in new geographic areas (Fanelli and Buonavoglia 2021). The expanding distribution of its main tick vector requires ongoing surveillance (Grandi et al., 2020). Whether shrew-derived nairoviruses depend on vector-borne transmission remains to be assessed, although the detection of RASV in *S. etruscus* from a breeding colony already established in 2005 (Geyer et al., 2022) raises doubts. Species-specific RT-qPCRs demonstrate high viral loads with a broad organ distribution. The highest concentrations were seen in well-perfused organs such as liver and heart.

Whole genomes of BoDV-1, another member of the order *Mononegavirales*, were determined in four *C. leucodon*, the known reservoir host, and additionally in two *C. suaveolens* and one *C. russula*. Viral RNA presence was independently confirmed using a BoDV-1-specific RT-qPCR assay (Schlottau et al., 2018). Thereby, this study represents the first report of BoDV-1 in closely related white-toothed shrews, which expands the potential reservoir spectrum.

Human hepatitis E virus causes self-limiting hepatitis in immunocompetent humans, which may become chronic in immunosuppressed patients (Velavan et al., 2021). It is transmitted via contaminated water and the consumption of raw animal products. It is a major health issue especially in resource-limited regions with low sanitary standards (WHO 2023). A novel hepevirus tentatively named *Paslahepevirus crocidurae* (shrewHEV) was identified in *C. russula* from two trapping sites across Germany. The new hepevirus is phylogenetically closer related to HEV than to rat hepatitis E virus (ratHEV), which was previously identified in Norway rats (*Rattus norvegicus)* (Johne et al., 2010), and later on also in black rats (*Rattus rattus*) (Ryll et al., 2017). Detections of ratHEV in *Suncus murinus* are most likely due to spillover from sympatric rats (Guan et al., 2013; Bai et al., 2020; He et al., 2018).

Interestingly, several co-detections of representatives of the four virus families were seen and their interactions need to be evaluated in the future. These co-infections may allow to postulate white-toothed shrews as hyperreservoirs. Of note, in none of the investigated samples any codetection with non-viral pathogens investigated in **Publication 1** were seen.

Reservoir-host-pathogen interactions depend on the biological properties of the reservoir as much as on the biological properties of the pathogen. Further studies are needed to determine whether shrews fulfil all requirements of a genuine reservoir, such as lifelong persistence and multiplication. Evaluation of Koch's postulates is fundamental towards assessing pathogenicity for other animals and humans (Fredricks and Relman 1996). The health status of the shrews prior to death could not be assessed as they were sent in dead. A negative impact of the viruses onto the white-toothed shrew populations cannot be excluded and need to be considered. Are the novel viruses causing any lesion in the shrews, or are they changing their behaviour making them easier prey?

Estimating the shrew-human interface is difficult. Shrews tend to seek shelter of human settlements particularly in temperate regions at higher altitudes (Kraft 2008), but are rarely recognized due to their elusive and skittish behaviour. Spatial overlap between species such as with synanthropic shrews and humans, lead to higher numbers of interspecific contacts, which is an essential driver for spillover (Dharmarajan et al., 2022; Ecke et al., 2022). It is necessary to assess pathogen diversity to establish recommendations on personal protective equipment for occupational groups such as small mammal biologists and ecologists. Additional shrew-human interfaces are created by handling of shrew carcasses preyed upon and displayed by cats and increased public awareness should be aimed for to reduce risks of transmission.

A One Health approach for the characterization of white-toothed shrews as reservoirs in the context of the first cluster of human BoDV-1 infection.

Publication 2, Publication 3, Review 2

Efforts have been devoted to comprehend the epidemiology of human BoDV-1 infections since BoDV-1 was identified as a zoonotic agent that causes fatal encephalitis in humans (Korn et al., 2018; Schlottau et al., 2018; Niller et al., 2020; Pörtner et al., 2023). Numerous questions remain unanswered regarding the stability and transmission of the pathogen, the spread of the pathogen within the organism, the duration of the incubation period, the range of clinical signs, and treatment options. Interviews with relatives of deceased patients were used to identify disease patterns, but is challenged by the low number of (reported) cases (Pörtner et al., 2023). With the detection of the first BoDV-1 infection cluster within a three-year window (Grosse et al., 2023), we were able to conduct an integrative and holistic One Health study in the respective municipality. This study targeted the serological status of the local human population, evaluated potential risk scenarios and underlying clinical symptoms by a questionnaire, examined the role of the environment and its reservoir in the transmission, and ruled out vector-borne transmission by investigating ticks.

The majority of acute cases have been admitted to hospitals and diagnosed only after the onset of severe symptoms (Niller et al., 2020; Grosse et al., 2023), and it still remains unknown whether oligosymptomatic and/or asymptomatic cases occur. In the field of serology, the labour-intensive IFAT is considered the gold standard (Rubbenstroth et al., 2019), but new tests are being developed e.g. enzyme-linked immunosorbent assay (ELISA) systems for reactive antibodies against BoDV-1 N, X, and P protein (Neumann et al., 2022) and IFN-γ-based-ELISpot (interferon-γ-based-enzyme-linked immunosorbent spot) assay to evaluate T-cell response (Eidenschink et al., 2023). Neither IFAT (n=679) nor IFN-γ-based-ELISpot assay (n=23) resulted in any detection of a seropositive citizen, indicating the absence of asymptomatic cases. Previous sero-epidemiological studies in blood donors, veterinarians, household members of BoDV-1 patients and with close contact to BoDV-1 positive animals, as well as neuropsychiatric patients and patients submitted for TBEV diagnostics, all within and outside the BoDV-1 endemic region, have demonstrated a very low seroprevalence (< 0.1%) (Tappe et al., 2019; Bauswein et al., 2023; Allartz et al., 2023). Those results underpin the severity of the disease with infected humans developing encephalitis, and asymptomatic/oligosymptomatic cases with seroconversion being rare events.

Infection of dead-end hosts with BoDV-1 may occur through direct contact to shrews, or indirect transmission through vectors, or contaminated environment (**Figure 3**). Due to the rare occurrence of BoDV-1 infections, as well as no reports on tick bites prior to infection (Pörtner et al., 2023), vector-borne transmission seemed unlikely, but needed to be further investigated. Previous BoDV-1

infection experiments of *Ixodes ricinus* failed (Schindler 2004). In line, all ticks (n=336) collected from two different sites tested negative for BoDV-1-RNA. Possible indirect transmission via the environment (soil, water, plants) possibly contaminated with shrew excreta was investigated by testing environmental samples (n=38), but neither the presence of infectious BoDV-1 nor its respective RNA was detected. Within an initial study, BoDV-1 remained infectious after up to four days of desiccation at ambient temperature (**Publication 3**). However, our findings do not exclude the presence of BoDV-1 in the environment, as the tenacity of BoDV-1 needs to be further evaluated and the choice of sampling sites and method need to be optimized, pending on the increasing knowledge of BoDV-1 presence in white-toothed shrews in the municipality.

In close collaboration with the local health department and the community itself, small mammals which were either preyed by cats or found dead due to unknown reason, were collected and tested for BoDV-1 RNA. In total, 43% of all investigated *C. leucodon* tested positive for BoDV-1-RNA confirming the circulation of the virus in the local shrew population. Interestingly, phylogenetic analysis of the human and shrew derived BoDV-1 sequences revealed two distinct subclades of subcluster 1A endemic to southern Bavaria and Baden-Wuerttemberg (**Figure 7**) (Kolodziejek et al., 2005). The BoDV-1 sequence from the first human patient, a 11-year-old girl who died in 2019, had a 99.1% pairwise nucleotide sequence identity to one of the shrew-derived BoDV-1 sequences. Since this specimen was caught by a cat, the precise range of the shrew is not available, and the cat owner lived in a stand-alone location at the outer edge of the municipality. All other BoDV-1 sequences of positive *C. leucodon* from the village were unambiguously linked to the second human case, a six-year-old boy, who died in 2022 (Grosse et al., 2023). If the first case got infected outside the community or if two parallel BoDV-1 strains are circulating within the local shrew population, potentially increasing the infection pressure, needs to be further evaluated. The detection rate in *C. leucodon* is within the range of previous studies conducted in the vicinity of animal Borna disease cases. However, it is challenging to compare one another and draw conclusions due to the different numbers of investigated animals, the varying sampling methods and the time frame. To comprehend the pathogen dynamics within the shrew population, a more systematic and longitudinal study of the shrew population is needed.

This is up to date the most comprehensive study on the shrew population in close proximity to any BoDV-1 case and provides us with valuable information on the overall composition of the shrew community and the detection rate of BoDV-1 within shrews. Although almost half of all investigated *C. leucodon* tested positive for BoDV-1-RNA, those represent only 7.7% of all investigated shrews. No spillover to any sympatric species was observed. This study provided valuable information on the overall species composition, with white-toothed shrews making up only 12% of all investigated shrews, after *Neomys* spp. (17%) and *Sorex* spp. (71%). This low number should be considered, when evaluating the infection risk and may be an explanation for the low BoDV-1 infection rate of dead-end hosts. Also

for domestic animals, BoDV-1 infection is a rare event (Weissenböck et al., 1998a; Caplazi et al., 1999; Jacobsen et al., 2010; FLI 2022). Only multiple infections in the same herd within a reasonable time frame have been observed for alpacas (Schulze et al., 2020). Alpacas and other New World camelids seem to be more susceptible to BoDV-1 than other mammal species, which indicates them as suitable sentinels (Jacobsen et al., 2010; Fürstenau et al., 2023; Malbon et al., 2022). The unique biological characteristic of BoDV-1 to replicate in the nucleus of the host cell provides another barrier for easy cross-species transmission. BoDV-1 was before only once detected in another shrew species, in a *Sorex araneus*, from the same area as BoDV-1-RNA positive *C. leucodon* in Austria (Weissenböck et al., 2017). This animal had a viral RNA tissue distribution equivalent to the exclusively neuronal BoDV-1 distribution pattern of dead-end hosts (Fürstenau et al., 2023; Liesche et al., 2019). This is in contrast to *C. leucodon*, which exhibit a broad viral RNA tissue distribution that allows excretion via multiple routes (urine, saliva, faeces, skin shedding) (Bourg et al., 2013; Nobach et al., 2015). We describe for the first time the detection of BoDV-1 in additional white-toothed shrews, in the greater and lesser white-toothed shrew (**Publication 2**), but no individual of the two species was trapped in the municipality. If those were spillover infections or if they are suitable reservoirs, expanding the host range of BoDV-1 has to be further evaluated. Sympatric appearance of the three species is rare as have the results of **Publication 1** demonstrated. The currently known endemic area of BoDV-1 is restricted to small regions and reported human and animal cases are either from or have a travel history from the known endemic areas (Priestnall et al., 2011; Tappe et al., 2021; Frank et al., 2022). The detection of BoDV-1 in additional species raises the question if BoDV-1 has the ability to drive in regions, where *C. leucodon* is absent from. To further determine the whole reservoir spectrum, sympatric species at sites with proven BoDV-1 presence, and white-toothed shrews from neighbouring countries should be investigated for the presence of BoDV-1 and unknown orthobornaviruses. For encephalitis cases of unknown origin BoDV-1 should be considered and tested for even in non-endemic regions. Participants enrolled in the questionnaire (n=679) had contact to shrews (19%) or shrew droppings (23%). Relatives of diseased patients reported no direct contact to shrews (biting, scratching) prior to infection (Pörtner et al., 2023), which seems to be a rare but not impossible event due to the shrews' elusive and skittish behaviour. Rural living conditions and potential exposure to the reservoir were identified as main risk factors for BoDV-1 infection (Niller et al., 2020; Pörtner et al., 2023; Bauswein et al., 2023), **Publication 3**). To prevent infection, further education should be provided on the safe handling of small mammal carcasses and on the use of personal protective equipment during high-risk activities (RKI 2020).

Concluding remarks

The results of this thesis shed light on the role of white-toothed shrews played in pathogen diversity, evolution and transmission. This study demonstrated a great pathogen diversity in just four of all recent white-toothed shrew species, representing only ~ 10% of globally distributed extant crocidurine species (**Publication 1** and **2**). Their elusive behaviour makes it difficult to estimate the exact human-shrew interface, which presumably exists due to their synanthropy. Pathogen spillover from white-toothed shrews to humans may occur, as evidenced by human BoDV-1 infections (**Publication 3**), but remain sporadic events.

Information on the exact shrew distribution, its' population density, and the composition of the small mammal community are needed to calculate infection risks (**Publication 1** and **2**). The current distribution of white-toothed shrews with regional preferences could be identified and sympatric occurrence of *C. leucodon* and *C. suaveolens* are more frequent than those of *C. russula* and *C. suaveolens* (**Publication 1**). Geographic proximity is next to phylogenetic relatedness a driver for cross-species transmission (Dharmarajan et al., 2022). Essentially, the greatest concern lies in the pathogen shedding and transmission to other animals. However, this depends not only on the pathogen-reservoir system, but also on the availability of susceptible hosts. Anthropogenic land use, biodiversity and climate change will most likely have an influence on the distribution of species and their associated infection risks, as species track-change climate conditions and invade new habitats (Daszak et al., 2001; Plowright et al., 2021; Carlson et al., 2022). Although this depends as much from extrinsic factors (passive movements by humans and predators, land use and climate change), as well from intrinsic dispersal abilities (home range, movement), which are rather low for the small sized non-migratory shrews.

Susceptibility and adverse strategy of the host system, including host immunology, host tolerance, and adaptability, determine the establishment of a robust pathogen-reservoir-host-system (Mandl et al., 2015). Currently, limited information on the immune system of white-toothed shrews are available. Trade-offs between a fast life cycle and a high metabolism with immunological properties have been described (Klein 2000), which may be one explanation for the high tolerance of bats towards multiple pathogens (Irving et al., 2021). It would be fascinating to investigate if similar effects occur in shrews. Models predicting new reservoir species have identified traits of an *r*-reproductive strategy as key factors for being a (hyper)reservoir (Han et al. 2015). Inter-species transmission must be very efficient to infect immune naïve populations and keep pathogen persistence in the population erect (Plowright et al., 2016). How pathogens are maintained in the shrew populations and how they are transmitted to other populations need to be further studied (**Publication 3**). Aggregation in large numbers benefits pathogen maintenance within a population, which is in contrast to the small family-based social structure of territorial shrews.

Our proactive screenings for novel viruses and neglected pathogens provide the advantage of being a step ahead of disease emergence, and enables to either prevent infection in the first place or at least be well prepared to take adequate precautions and countermeasures (**Publication 1** and **2**).

An increase in outdoor recreation (Beery et al., 2021) and subsequent potential interactions with local wildlife has been observed, which is positive as it raises awareness for nature and wildlife conservation. However, increased contact may also pose risks, especially for children and elderly who may tend to spend more time outdoors. Since they may have weakened immune systems, they could face a greater vulnerability towards acquiring zoonotic diseases (Wolfe et al., 2007; CDC 2021). Testing of risk groups that are potentially in contact with shrews, such as agricultural workers, is recommended to determine presence or absence of the novel shrew-borne viruses in the human population (**Publication 2**). This study has demonstrated a unique white-toothed shrew virome as the shrew-borne viruses form their own phylogenetic clades distinct from those of other small mammals (**Publication 2**).

The impact on the conservation of species should be considered when performing pathogen discovery studies (Daszak et al., 2000; Breed et al., 2006; Sokolow et al., 2019). The most effective way to prevent potential spillover is to reduce the interface between humans and wildlife, which is difficult in synanthropic animals. To develop appropriate management plans to achieve sustainable co-existence with a minimized infection risks (for humans) without negatively impacting the shrew population requires a multidisciplinary team (**Publication 3**) (Sokolow et al., 2019).

Further understanding of the factors that play a role in spillover of pathogens from shrews to animals and humans, require structured, systematic, longitudinal surveillance of their populations that includes multi- and transdisciplinary approaches fulfilling the One Health concept.

VI. Summary

In this thesis, the role of European white-toothed shrews as reservoirs for various known and previously unknown pathogens was investigated. A "reservoir" is defined as an animal that reproduces and excretes a pathogen without succumbing to disease. Shrews are a phylogenetically ancient, globally distributed and species-rich group. Three subfamilies are distinguished: red-toothed shrews (Soricinae), white-toothed shrews (Crocidurinae) and African white-toothed shrews (Myosoricinae). The three white-toothed shrew species present in Central Europe, namely the lesser white-toothed shrew (*Crocidura suaveolens*), bicolored white-toothed shrew (*Crocidura leucodon*) and greater white-toothed shrew (*Crocidura russula*) were studied together with the smallest recent shrew species, the Etruscan shrew (*Suncus etruscus*), which occurs in the Mediterranean region and is hold in captivity in Germany. The collection of shrews from Germany and additional European countries enabled a better portrayal of their current distribution, which is subject to fluctuations and only incompletely described. *Leptospira kirschneri* was detected in *C. russula* and *C. leucodon* and *Neoehrlichia mikurensis*-DNA was obtained from *C. russula* for the first time. The absence of *Coxiella burnetii*, *Brucella* spp. and particularly arthropod-borne pathogens (*Anaplasma phagocytophilum*, *Bartonella* spp., and *Babesia* spp.) may indicate a minor role of white-toothed shrews in the transmission cycle of these pathogens possibly due to a shrew-specific ectoparasite milieu.

The virome of crocidurine shrews from Central Europe has not been studied previously. To characterise the virome, samples of the four crocidurine shrew species were analysed using high-throughput sequencing. Several complete genomes of hitherto unknown viruses of the families *Paramyxoviridae*, *Nairoviridae* and *Hepeviridae* were detected. These viruses are phylogenetically closely related to globally occurring pathogens with health risks for humans and animals. In particular, the Hasua virus (*Paramyxoviridae*) is noteworthy due to its phylogenetic proximity to the zoonotic Langya virus, recently identified in China. In addition, the presence of Borna disease virus 1 (BoDV-1) was detected for the first time in *C. russula* and *C. suaveolens*. Co-infections with up to three of the discovered viruses were found in several shrews; co-infections with the bacterial pathogens were not observed. Further research is required to determine the pathogenicity and zoonotic potential of the virus species discovered in this study.

Extensive One Health-concept based investigations were carried out in the municipality with the first human BoDV-1 cluster. No anti-BoDV-1-antibodies or BoDV-1-RNA could be detected in any of the human serum or nasopharyngeal swab samples, environmental samples and ticks. The resident's questionnaire revealed likely contact to shrews and their excreta. An established 'Citizen Science'-project enabled the investigation of shrews and other small mammals. However, the presence of BoDV-1 was exclusively detected in *C. leucodon*. Different BoDV-1 variants, closely related to the BoDV-1 sequences obtained

from the deceased children, were phylogenetically identified in the municipality's *C. leucodon* population. Thus, the presence of BoDV-1 and an existing shrew-human interface could be confirmed beyond doubt. Future longitudinal studies will provide important information on the stability and transmission of BoDV-1, which can be used for recommendations of preventive measures. Our data, together with additional multidisciplinary studies within the One Health-concept assessing the biology and ecology of crocidurine shrews, will provide guidance to fully comprehend the shrew-human interface and to promote sustainable co-existence with these elusive, synanthropic species.

VII. Zusammenfassung

In dieser Dissertation wurde die Bedeutung von europäischen Weißzahnspitzmäusen als Reservoire für verschiedene bekannte und bisher unbekannte Pathogene untersucht. Als „Reservoir“ wird in der Regel ein Tier definiert, das einen Erreger vermehrt und ausscheidet, ohne selbst an der Infektion zu erkranken. Spitzmäuse sind eine phylogenetisch alte, weltweit vorkommende, artenreiche Gruppe. Es wird zwischen drei Unterfamilien unterschieden: den Rotzahnspitzmäusen (Soricinae), den Weißzahnspitzmäusen (Crocidurinae) und den Afrikanischen Weißzahnspitzmäusen (Myosoricinae). Neben den drei in Mitteleuropa heimischen Weißzahnspitzmaus-Arten, Gartenspitzmaus (*Crocidura suaveolens*), Feldspitzmaus (*Crocidura leucodon*) und Hausspitzmaus (*Crocidura russula*), wurde die kleinste Spitzmausart, die Etrusker Spitzmaus (*Suncus etruscus*), die im Mittelmeerraum vorkommt und in Deutschland in Gefangenschaft gehalten wird, mit einbezogen. Die Probensammlung in Deutschland und Nachbarländern ermöglichte eine verbesserte Beschreibung der aktuellen Verbreitung der Arten, die zum Teil nur lückenhaft bekannt ist und Schwankungen unterliegt. Erstmalig wurde *Neoehrlichia mikurensis*-DNA in Hausspitzmäusen nachgewiesen. Die Untersuchungen führten zum Nachweis von *Leptospira kirschneri* in Haus- und Feldspitzmäusen. Der fehlende Nachweis von *Coxiella burnetii, Brucella* spp., und vor allem von Arthropoden-übertragenen Erregern (*Anaplasma phagocytophilum, Bartonella* spp., und *Babesia* spp.) deutet möglicherweise auf eine geringe Rolle der Weißzahnspitzmäuse im Übertragungszyklus von diesen Erregern hin, was auf ein spitzmausspezifisches Ektoparasiten-Milieu zurückzuführen sein könnte.

Wenig ist über die bei Weißzahnspitzmäusen in Mitteleuropa vorkommende Gesamtheit der Viren, das Virom, bekannt. Zur Charakterisierung des Viroms wurden Proben der oben genannten Spitzmausarten mittels Hochdurchsatzsequenzierung untersucht. Mehrere Komplettgenome von bisher unbekannten Viren der Familien *Paramyxoviridae, Nairoviridae* und *Hepeviridae* wurden nachgewiesen, die phylogenetisch nahe verwandt sind zu global vorkommenden Erregern mit Gesundheitsrisiko für Mensch und Tier. Insbesondere das hier entdeckte Hasua-Virus aus einer Gartenspitzmaus sticht durch seine phylogenetische Nähe zu dem kürzlich in China beschriebenen zoonotischen Langya-Virus hervor. Darüber hinaus konnte das Vorkommen des Borna disease virus 1 (BoDV-1) erstmalig in Haus- und Gartenspitzmäusen, sowie in dem bekannten Reservoir, der Feldspitzmaus, nachgewiesen werden. Bei mehreren Spitzmäusen wurden Ko-infektionen mit bis zu drei der genannten Erreger gefunden; Ko-infektionen mit den bakteriellen Erregern wurden nicht beobachtet. Weitere Untersuchungen sind notwendig, um das zoonotische Potenzial der hier entdeckten Virusspezies zu identifizieren.

Im Zusammenhang mit dem ersten humanen BoDV-1-Cluster wurden in der betroffenen Gemeinde umfangreiche One Health-Konzept-basierte Untersuchungen durchgeführt. In keiner der humanen

Serum- bzw. Nasentupfer-Proben, Umweltproben und Zecken konnten anti-BoDV-1-Antikörper beziehungsweise BoDV-1-RNA nachgewiesen werden. Die Befragung der Anwohner ergab, dass ein hoher Anteil möglichen Kontakt zu Spitzmäusen und deren Ausscheidungen hatte. Ein etabliertes „Citizen Science“-Projekt ermöglichte die Untersuchung von Spitzmäusen und anderen Kleinsäugern. BoDV-1 wurde exklusiv in Feldspitzmäusen nachgewiesen. Phylogenetische Untersuchungen zeigten, dass in der Feldspitzmauspopulation der Gemeinde zwei unterschiedliche BoDV-1-Varianten vorkommen, die nahe mit den bei den verstorbenen Kindern gefundenen BoDV-1-Sequenzen verwandt sind. Somit konnte in der Gemeinde das Vorkommen von BoDV-1, sowie ein bestehendes Spitzmaus-Mensch-Interface zweifelsfrei bestätigt werden. Zukünftige Langzeitstudien werden wichtige Informationen zu Stabilität und Übertragung von BoDV-1 in der lokalen Spitzmauspopulation liefern, um darauf basierend Empfehlungen zur Infektionsvermeidung für die Bevölkerung zu erstellen. Ergänzende Untersuchungen zur Biologie und Ökologie der Spitzmausarten sind notwendig, um unter Einbeziehung der hier gewonnenen Erkenntnisse zur Pathogenvielfalt, Handlungsempfehlungen zum sicheren Umgang mit Spitzmäusen zu erstellen, die ein nachhaltiges Zusammenleben zwischen Mensch und Spitzmaus ermöglichen.

VIII. References

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IX. Appendix

9.1 List of Figures

9.2 List of Tables

9.3 Legal permissions

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Figure 9 Alpaca picture obtained from https://www.phylopic.org/, 20.09.2023.

9.4 Abbreviations

BSL 4	Biosafety level 4
B. P.	Before the present
cDNA	copy DNA
contigs	Paired sequence reads
CSF	Cerebrospinal fluid
ct-value	Cycle threshold value
DNA	Deoxyribonucleic acid
EBL	Endogenous bornavirus-like genetic element
itEBLN-1	N-encoding EBL from a thirteen-lined ground squirrel
EIDs	Emerging infectious diseases
ELISA	Enzyme-linked immunosorbent assay
TEM	Transmission electron microscopy
FGS	First-generation sequencing
G	Surface glycoprotein
H.E. staining	Hematoxylin-eosin staining
GISD	Global Invasive Species Database
(m) HTS	(Metagenomic) high-throughput sequencing
ICTV	International Committee on Taxonomy of Viruses
IFAT	Immunofluorescence antibody test
IfSG	Infektionsschutzgesetz
IFN-γ-based-ELISpot	Interferon-γ-based-enzyme-linked immunosorbent spot
L	Large protein
LM	Light microscopy
locus	Housekeeping gene
kB	Kilobases
M	Matrix protein
MLST	Multi-locus sequence typing
Myr	Million years
N	Nucleoprotein
ONT	Oxford Nanopore Technology
ORF	Open reading frame
P	Phosphoprotein
PASC	Pairwise Sequence Comparison
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
cRNA	complementary positive-stranded RNA
mRNA	messenger RNA
(-) ssRNA	Negative-sense, single-stranded RNA
RdRp	RNA-directed RNA polymerase
(RT-) qPCR	quantitative real-time (reverse transcription) polymerase chain reaction
secY	Transmembrane protein
SGS	Second-generation sequencing
SLST	Single-locus sequence typing
ST	Sequence type
TGS	Third-generation sequencing
vCJD	variant Creutzfeld-Jakob disease
WGS	Whole-genome sequencing
WHO	World Health Organization
X	Accessory protein

9.5 Taxonomy

Table 5 Taxonomy of all mentioned virus species in this thesis according to the current International Committee on Taxonomy of Viruses and alphabetically ordered by the virus species abbreviation.

Order	Genus	Species	Common name	Abbreviation
Asfarviridae	*Asfivirus*	*African swine fever virus*	African swine fever virus	ASFV
Bornaviridae	*Orthobornavirus*	*Orthobornavirus bornaense*	Borna disease virus 1	BoDV-1
Nairoviridae	*Orthonairovirus*	*Orthonairovirus haemorrhagiae*	Crimean-Congo haemorrhagic fever virus	CCHFV
Nairoviridae	*Orthonairovirus*	not ICTV approved yet	Cencurut virus	CENV
Paramyxoviridae	*Morbillivirus*	*Morbillivirus canis*	canine distemper virus	CDV
Paramyxoviridae	*Henipavirus*	not ICTV approved yet	Denwin virus	DewV
Bunyaviridae	*Orthohantavirus*	*Dobrava-Belgrade orthohantavirus*	Dobrava virus	DOBV
Nairoviridae	*Orthonairovirus*	*Orthonairovirus dugbeense*	Dugbe virus	DUGV
Filoviridae	*Ebolavirus*	*Orthoebolavirus zairense*	Ebola virs	EBOV
Nairoviridae	*Orthonairovirus*	*Orthonairovirus erveense*	Erve virus	ERVEV
Paramyxoviridae	*Henipavirus*	not ICTV approved yet	Gamak virus	GamV
Paramyxoviridae	*Henipavirus*	this thesis	Hasua virus	HasV
Nairoviridae	*Orthonairovirus*	*Orthonairovirus hazaraense*	Hazara virus	HAZV
Flavivirdae	*Hepacivirus*	*Hepacivirus hominis*	hepatitis C virus	HCV
Paramyxoviridae	*Henipavirus*	*Henipavirus hendraense*	Hendra virus	HeV
Hepeviridae	*Paslahepevirus*	*Paslahepevirus balayani*	human hepatitis E virus	HEV
Retroviridae	*Lentivirus*	*human immunodeficiency virus 1*	human immunodeficiency virus 1	HIV-1
Nairoviridae	*Orthonairovirus*	*Orthonairovirus issykkulense*	Issy-kul virus	ISKV
Nairoviridae	*Orthonairovirus*	not ICTV approved yet	Lamgora virus	LMGV
Nairoviridae	*Orthonairovirus*	not ICTV approved yet	Lamusara virus	LMSV
Paramyxoviridae	*Henipavirus*	*Langya virus*	Langya virus	LayV
Arenaviridae	*Mammarenavirus*	*Mammarenavirus lassaense*	Lassa virus	LASV
Arenaviridae	*Mammarenavirus*	*Mammarenavirus choriomeningitidis*	lymphocytic choriomeningitis virus	LCMV
Paramyxoviridae	*Henipavirus*	this thesis	Lechcodon virus	LechV
Filoviridae	*Marburgvirus*	*Orthomarburgvirus marburgense*	Marburg virus	MARV
Paramyxoviridae	*Morbillivirus*	*Morbillivirus hominis*	measles virus	MeV
Paramyxoviridae	*Henipavirus*	*Henipavirus mojiangense*	Mòjiāng virus	MojV
Poxviridae	*Orthoposvirus*	*Monkeypox virus*	monkey pox virus	MPV
Paramyxoviridae	*Orthoavulavirus*	*Avian orthoavulavirus 1*	Avian paramyxovirus 1/ Newcastle disease	APMV-1/ NDV
Paramyxoviridae	*Henipavirus*	*Henipavirus nipahense*	Nipah virus	NiV
Nairoviridae	*Orthonairovirus*	*Orthonairovirus nairobiense*	Nairobi Sheep disease virus	NSDV
Bunyaviridae	*Orthohantavirus*	*Puumala orthohantavirus*	Puumala virus	PUUV
Rhabdoviridae	*Lyssavirus*	*Lyssavirus rabies*	rabies virus	RABV
Nairoviridae	*Orthonairovirus*	this thesis	Rasenna virus	RASV
Hepeviridae	*Rocahepevirus*	*Rocahepevirus rattii*	rat hepatitis E virus	ratHEV

Table to be continued.

Order	Genus	Species	Common name	Abbreviation
Nairoviridae	*Orthonairovirus*	this thesis	Regana virus	REGV
Paramyxoviridae	*Henipavirus*	this thesis	Resua virus	ResV
Paramyxoviridae	*Morbillivirus*	*Morbillivirus pecoris*	rinderpest virus	RPV
Matonaviridae	*Rubivirus*	*Rubivirus rubella*	rubella virus	RuV
Coronaviridae	*Betacoronavirus*	*Severe acute respiratory syndrome-related coronavirus*	severe acute respiratory syndrome coronavirus	SARS-CoV
Coronaviridae	*Betacoronavirus*	*Severe acute respiratory syndrome-related coronavirus*	severe acute respiratory syndrome coronavirus 2	SARS-CoV-2
Paramyxoviridae	*Respirovirus*	*Respirovirus muris*	Sendai virus	SeV
Bunyaviridae	*Orthohantavirus*	*Seoul orthohantavirus*	Seoul virus	SEOV
Polyomaviridae	*Polyomavirus*	*Simian virus 40*	simian virus 40	SV 40
Flaviviridae	*Orthoflavivirus*	*Orthoflavivirus encephalitidis*	Tick-borne encephalitis virus	TBEV
Nairoviridae	*Orthonairovirus*	*Orthonairovirus thiaforaense*	Thiafora virus	TFAV
Poxviridae	*Orthopoxvirus*	*Variola virus*	variola virus	VARV
Bornaviridae	*Orthobornavirus*	*Orthobornavirus sciuri*	variegated squirrel bornavirus 1	VSBV-1
Flaviviridae	*Orthoflavivirus*	*Orthoflavivirus nilense*	West Nile virus	WNV

Table 6 Taxonomy of mentioned animal species in this thesis according to Wilson and Reeder 2005 and Wilson and Mittermaier 2017.

Order	English name	Latin name	Described by
Strigiformes	barn owl	*Tyto alba*	(Scopoli, 1769)
Chiroptera	hog-nosed bat	*Craseonycteris thonglongyai*	Hill, 1974
Carnivora	African lion	*Panthera leo*	Linnaeus, 1758
Rodentia	Prevost's squirrel	*Callosciurus prevostii*	(Demarest, 1822)
	thirteen-lined ground squirrel	*Ictidomys tridecemlineatus*	(Mitchill, 1821)
	variegated squirrel	*Sciurus variegatoides*	Ogilby, 1839
	Natal mastomys	Mastomys natalensis	(Smith,1834)
	field vole	*Microtus agrestis*	(Linnaeus, 1761)
	house mouse	*Mus musculus*	Linnaeus, 1758
	Norway rat	*Rattus norvegicus*	(Berkenhout, 1769)
	black rat	*Rattus rattus*	(Linnaeus, 1758)
Eulipotyphla	Western European hedgehog	*Erinaceus europaeus*	Linnaeus, 1758
	Northern white-breasted hedgehog	*Erinaceus roumanicus*	Barrett-Hammilton, 1900
	Asian gray shrew	*Crocidura attenuata*	Milne-Edwards, 1872
	large white-toothed shrew	*Crocidura dracula*	Thomas, 1912
	Doucet's musk shrew	*Crocidura douceti*	Heim de Balsac, 1958
	Dsinezumi shrew	*Crocidura dsinezumi*	(Temminck, 1842)
	greater red musk shrew	*Crocidura flavescens*	(I. Geoffrey, 1827)
	Gmelin's white toothed shrew	*Crocidura gmelini*	(Pallas, 1811)
	Goliath shrew	*Crocidura goliath*	Thomas, 1906
	large-headed shrew	*Crocidura grandiceps*	Hutterer, 1983
	lesser red musk shrew	*Crocidura hirta*	Peters, 1852
	Horsfield's shrew	*Crocidura horsfieldii*	(Thomas, 1856)
	Ussuri white-toothed shrew	*Crocidura lasiura*	Dobson, 1890
	bicolored white-toothed shrew	*Crocidura leucodon*	(Hermann, 1780)
	Niobe's shrew	*Crocidura obscurior*	Heim de Balsac, 1958
	African giant shrew	*Crocidura olivieri*	(Lesson, 1827)
	greater white-toothed shrew	*Crocidura russula*	(Hermann, 1780)
	Shantung white-toothed shrew	*Crocidura shantungensis*	Miller 1901
	Somali shrew	*Crocidura somalica*	Thomas, 1895
	lesser white-toothed shrew	*Crocidura suaveolens*	(Pallas, 1811)
	There's shrew	*Crocidura theresae*	Heim de Balsac, 1968
	lesser Ryukyu shrew	*Crocidura watasei*	Kuroda, 1924
	Etruscan shrew	*Suncus etruscus*	(Savi, 1822)
	Asian house shrews	*Suncus murinus*	(Linnaeus, 1766)
	Mediterranean water shrew	*Neomys anomalus*	Cabrera, 1907
	Eurasian water shrew	*Neomys fodiens*	(Pennant, 1771)
	Alpine shrew	*Sorex alpinus*	Schinz, 1837
	common shrew	*Sorex araneus*	Linnaeus, 1758
	Laxmann'shrew	*Sorex caecutiens*	Laxmann, 1788

Table to be continued.

Order	English name	Latin name	Described by
	Cinereus shrew	*Sorex cinereus*	Kerr, 1792
	crowned shrew	*Sorex coronatus*	Millet, 1828
	Siberian large-toothed shrew	*Sorex daphaenodon*	Thomas, 1907
	Eurasian least shrew	*Sorex minutissimus*	Zimmermann, 1780
	Eurasian pygmy shrew	*Sorex minutus*	Linnaeus, 1766
	Dusky shrew	*Sorex monticolus*	Merriam, 1890
	flat-skulled shrew	*Sorex roboratus*	Hollister, 1913
	Tundra shrew	*Sorex tundrensis*	Merriam, 1900
	long-clawed shrew	*Sorex unguiculatus*	Dobson, 1890

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