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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).

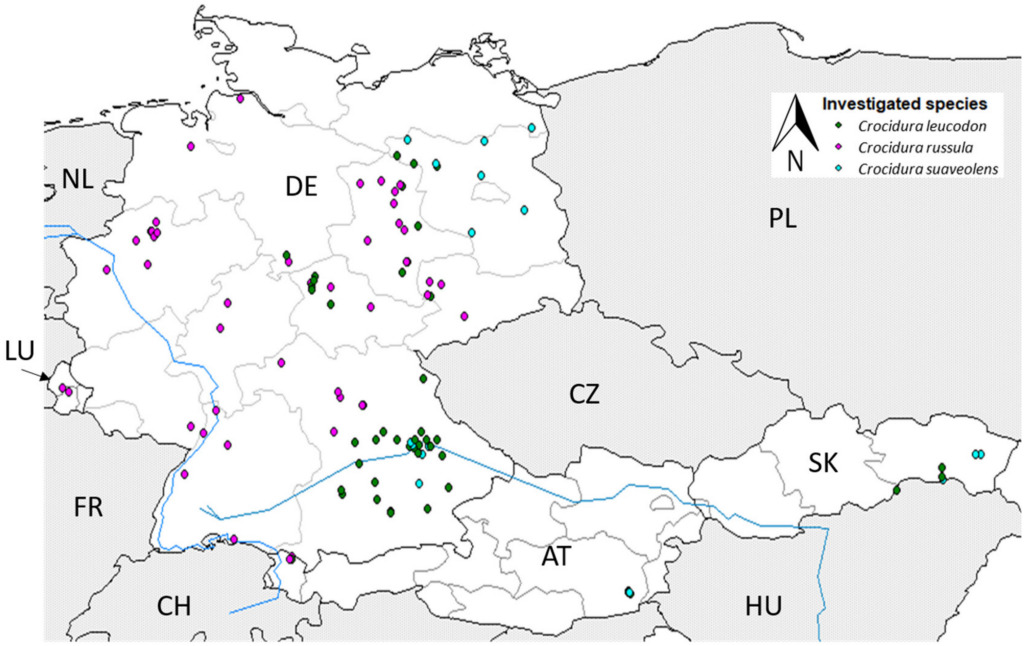

Figure 1. Origin of the investigated white-toothed shrews from Germany (n = 341), Luxembourg (n = 2), Austria (n = 18) and Slovakia (n = 11) based on common postal code; per trapping site, each detected species is represented by one dot. NL: the Netherlands; LU: Luxembourg; FR: France; DE: Germany; CH: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

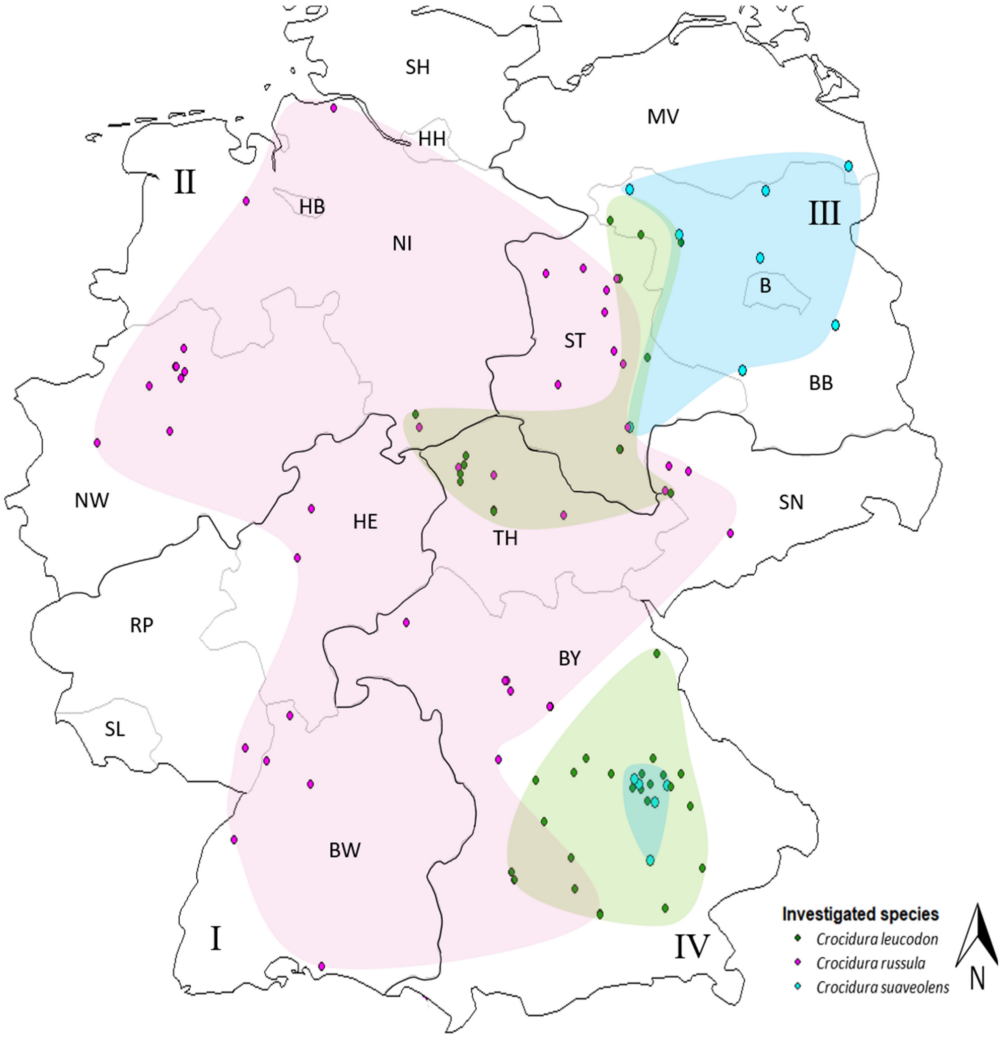

Figure 2. Distribution of investigated white-toothed shrews from Germany: greater white-toothed shrew (*Crocidura russula*, purple), bicolored white-toothed shrew (*Crocidura leucodon*, green), lesser white-toothed shrew (*Crocidura suaveolens*, blue); per trapping site, each detected species is represented 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).

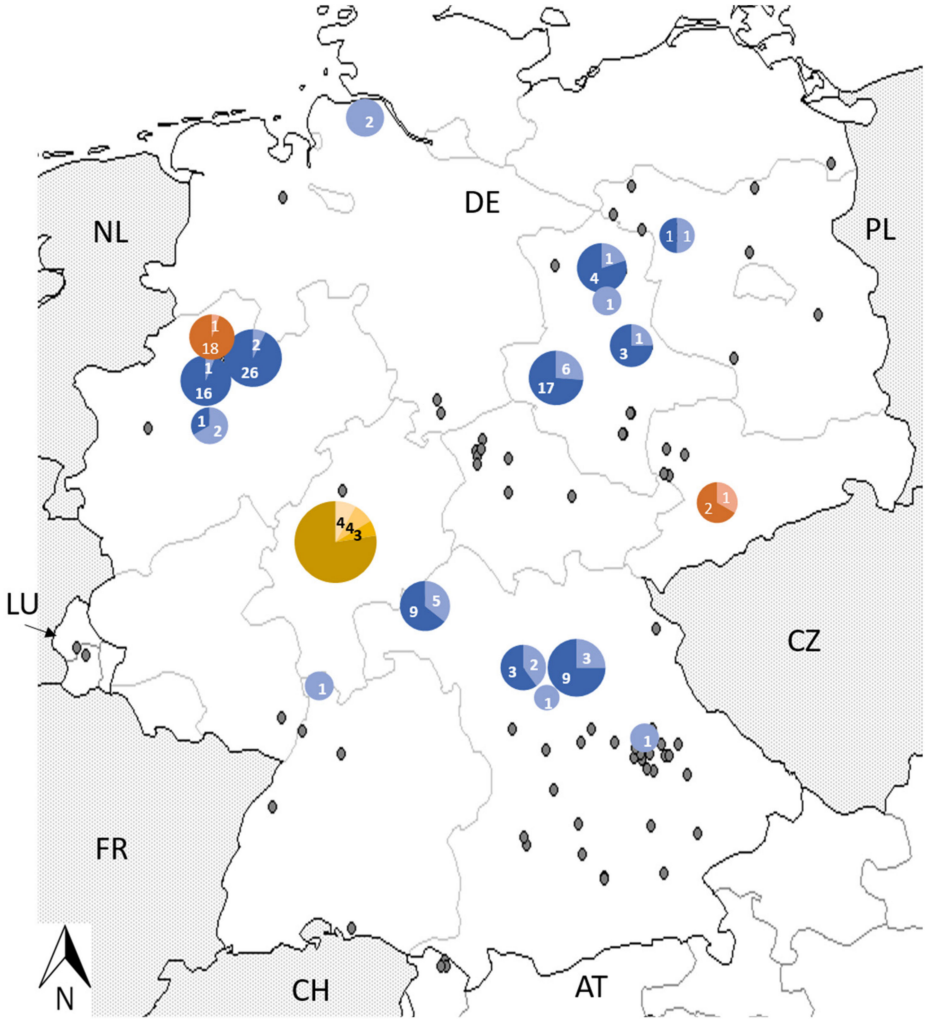

Figure 3. Detection of *Leptospira kirschneri* DNA (blue) and *Neoehrlichia mikurensis* DNA (orange) in white-toothed shrews. Numbers of positive individuals are indicated by a brighter colour. Trapping sites with no detection of any investigated pathogens are marked in grey. Investigations into hedgehogs 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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