Zoonoses and Public Health

WILEY

REVIEW OPEN ACCESS

Brucella microti and Rodent-Borne Brucellosis: A Neglected Public Health Threat

Ivo Rudolf[1,2] | Romana Kejíková[1] | Michael Kosoy[3] | Zdeněk Hubálek[1] | Kristína Mravcová[1] | Silvie Šikutová[1] | Adrian M. Whatmore[4] | Sascha Al Dahouk[5]

[1]Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic | [2]Department of Experimental Biology, Masaryk University, Brno, Czech Republic | [3]KB One Health, Fort Collins, Colorado, USA | [4]Department of Bacteriology, Animal & Plant Health Agency, Weybridge, UK | [5]Department 1 - Infectious Diseases, Robert Koch Institute, Berlin, Germany

Correspondence: Ivo Rudolf (rudolf@ivb.cz)

Received: 25 April 2024 | **Revised:** 1 October 2024 | **Accepted:** 4 October 2024

Funding: This work was financially supported by the Ministry of Health of the Czech Republic (Grant Number NU21-05-00143).

Keywords: *Brucella* | public health risk | rodent | sapronosis | zoonosis

ABSTRACT

Brucellosis is one of the most important zoonoses worldwide, primarily affecting livestock but also posing a serious threat to public health. The major *Brucella* species are known to cause a feverish disease in humans with various clinical signs. These classical *Brucella* species are (re-)emerging, but also novel strains and species, some of them transmitted from rodents, can be associated with human infections. As a result of our review on rodent-borne brucellosis, we emphasise the need for more comprehensive surveillance of *Brucella* and especially *Brucella microti* in rodent populations and call for further research targeting the ecological persistence of rodent-associated *Brucella* species in the environment, their epizootic role in wild rodents and their virulence and pathogenicity for wildlife.

1 | Introduction

Knowledge about the diversity of the genus *Brucella* has grown remarkably over the past 15 years, expanding our understanding of the genus (Whatmore and Foster 2021). Nowadays, the genus *Brucella* can be tentatively divided into six hitherto called "classical" *Brucella* species (*B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*), the marine mammal species (*B. pinnipedialis* and *B. ceti*) (Foster et al. 2007) and various novel species, such as *B. inopinata* initially isolated from a human breast implant wound (De et al. 2008; Scholz et al. 2010), *B. papionis* from baboons (Whatmore et al. 2014), *B. vulpis* from red foxes (Scholz et al. 2016b), *B. microti* from voles (Scholz et al. 2008b) and numerous strains awaiting taxonomic classification from frogs (Al Dahouk et al. 2017; Eisenberg et al. 2012; Fischer et al. 2012; Kimura et al. 2017; Mühldorfer et al. 2017; Scholz et al. 2016a; Soler-Lloréns et al. 2016), reptiles (Eisenberg et al. 2020), dogs (Guzmán-Verri et al. 2019), bats (Bai et al. 2017), rodents (Tiller et al. 2010a), humans (Tiller et al. 2010b) and fish (Eisenberg et al. 2017).

While a group of 10 species that have been described as "core" clade (i.e., the forementioned six "classical", the marine mammal [*B. pinnipedialis* and *B. ceti*] and two of the novel [*B. papionis* and *B. microti*]; Occhialini et al. 2022; Whatmore and Foster 2021) show very high genomic homogeneity and similarity (e.g., in 16S rRNA and *recA* gene sequences), some of the novel *Brucella* spp. (*B. vulpis* and *B. inopinata*) appear to be more heterogeneous ("non-core" clade) but are still embedded in the genus *Brucella* (Occhialini et al. 2022; Olsen and Palmer 2014; Scholz et al. 2016a; Whatmore 2009). Moreover, the comparison of phenotypic characteristics between classical and novel species has led some researchers to propose an adapted terminology referring to atypical phenotypic characteristics (diverse

Summary

- Brucellosis is one of the most important zoonoses worldwide which is frequently misdiagnosed due to its non-specific clinical signs and symptoms and because of imperfect diagnostic tests.
- The recent discovery of novel species and strains of *Brucella* (including rodent-borne variants) and the increasing number of human cases caused by them should prompt targeted monitoring and comprehensive surveillance in wildlife and the environment.
- Given the detection of *B. microti* in rodents and soil and the occurrence of *B. microti*-like strains in farmed frogs, awareness of the potential public health risk should be raised, especially among highly exposed groups such as farmers, hunters, forestry workers, zoologists as well as veterinarians.

LPS variants and motility, higher metabolic activity or modified metabolism and fast growth) labelled as "atypical." These atypical *Brucella* spp. include several members, namely *B. microti*, *B. inopinata* and other atypical *Brucella* that have emerged from cold-blooded animals (e.g., *B. inopinata*- and *B. microti*-like strains) (Occhialini et al. 2022). The taxonomy of the genus has faced another recent challenge after the proposal to reclassify *Ochrobactrum* spp. as members of the genus *Brucella* (Hördt et al. 2020; Moreno et al. 2022). To avoid confusion, the former name *Ochrobactrum* is used throughout our manuscript (Moreno et al. 2023).

The most relevant *Brucella* species have been known for almost 100 years to cause disease with variable clinical symptoms. Brucellosis is one of the most important zoonosis worldwide, primarily affecting livestock but also posing a serious threat to public health. Despite successful eradication of bovine and ovine/caprine brucellosis in many developed countries, the emergence of the novel *Brucella* should raise awareness in public health officers, general practitioners and clinicians (El-Sayed and Awad 2018; Zhang et al. 2018). Novel *Brucella* species associated with human disease include *B. inopinata*- (strain BO1) (De et al. 2008) and *B. inopinata*-like strain BO2 from lung biopsy of a patient with chronic destructive pneumonia (Tiller et al. 2010b). *B. ceti* (sequence type ST27) has also been considered a potential zoonotic agent, with three human cases reported to date (Whatmore et al. 2008)—two cases of neurobrucellosis with granuloma (Sohn et al. 2003) and one of spinal osteomyelitis (McDonald et al. 2006). The first case of human brucellosis caused by amphibian-type brucellae closely related to *B. inopinata*-like strain BO2 and isolates from American Pacman frogs (*Ceratophrys ornata*) was described in a person from France who had close occupational contact with exotic amphibian and reptile species (Rouzic et al. 2021). Recently, two human infections caused by novel *Brucella* species (not yet taxonomically assigned) were documented in goldminers working in French Guiana. Zoonotic transmission from a wildlife reservoir through the consumption of bushmeat (probably wild boar) was suspected (About et al. 2023).

2 | Occurrence of *Brucella* spp. in Rodent Populations

The occurrence of *Brucella* in rodent populations was reported in Africa (Heisch et al. 1963), Asia (Truong et al. 2011), Australia (Chakma et al. 2017; Cook, Campbell, and Barrow 1966; Tiller et al. 2010a), Europe (Dranovskaia, Malikov, and Grekova 1983; Hammerl et al. 2017; Hubálek et al. 2007; Scholz et al. 2008b; Vershilova et al. 1983) and North America (Boeer et al. 1980; Moore and Schnurrenberger 1981). Some of the isolates from rodents were historically identified as core *Brucella* clade, either as *B. abortus* (Boeer et al. 1980; Moore and Schnurrenberger 1981; Truong et al. 2011) or as *B. suis* (Cook, Campbell, and Barrow 1966; Dranovskaia, Malikov, and Grekova 1983; Heisch et al. 1963; Meyer 1976) but had to be reclassified with the introduction of molecular methods. To the best of our knowledge, there is only one study of brucellosis prevalence in rodents (Hammerl et al. 2017). The molecular survey revealed that 14.2% (76/537) of rodents and shrews sampled from four areas of Germany were infected by *Brucella*. Most of the animals detected as infected were rodents of the genus *Apodemus* and bank voles (*Myodes glareolus*). However, this study for the first time also described the presence of *Brucella* in shrews of the family *Soricidae*. These results support the hypothesis that *Brucella* spp. may be distributed not only in rodents but also in other wildlife species and environmental niches all over the world, as suggested (Pappas 2010). The epidemiological role of rodents as reservoirs remains unknown and needs to be addressed.

In the following sections, we will discuss the characteristics of classical *Brucella* spp. isolated from rodents, namely *B. suis* bv 5 and *B. neotomae*, as well as of the novel species, namely *B. vulpis* and *B. microti*.

3 | Classical *Brucella* spp.

3.1 | *Brucella suis* bv 5

Various strains obtained from rodents expressed phenotypic traits, distinguishing them from existing *Brucella* species and biovars. Such atypical isolates were found in striped field mice (*Apodemus agrarius*) and common voles (*Microtus arvalis*) captured in Northern Caucasus (Dushina et al. 1964). In-depth analysis of their cultural, biochemical and pathogenic properties demonstrated their unique characteristics. Though these strains were closer to *B. suis* than to other core *Brucella* spp., their capacity for agglutination with monospecific sera, their high sensitivity to pyronin B, safranin T and gentian violet, their low urease activity and their broad substrate oxidation activity pointed to a special taxonomic status (Liamkin et al. 1983; Liamkin, Tiumentseva, and Afanas'ev 1983; Vershilova et al. 1983). These isolates were eventually described as *B. suis* bv 5 (Corbel 1984) and the relationship as an early branching taxon in the *B. suis* lineage was confirmed by subsequent molecular analysis (Wattam et al. 2014). Although *B. suis* bv 5 has rarely been described since these initial studies there is some evidence for zoonotic potential. In 1993, the isolation of *B. suis*

bv 5 from a cat and a human contact diagnosed with brucellosis was reported (Repina, Nikulina, and Kosilov 1993), a frequently cited *B. suis* bv 5 strain (ELT80) is known to be isolated from a human brucellosis case (A.M. Whatmore, unpublished data) and, more recently, comprehensive phenotypic and molecular analysis confirmed a case of human brucellosis in Germany caused by *B. suis* bv 5 of unknown source (Brangsch, Horstkotte, and Melzer 2023).

3.2 | *Brucella neotomae*

The first *Brucella* species isolated from rodents was described in 1957 and was named *B. neotomae* after the genus of its rodent host, the desert woodrat (*Neotoma lepida*). These strains, recovered from tissue samples of desert woodrats captured in the Great Salt Lake Desert in the US State of Utah, differed in several phenotypic traits from other known *Brucella* species at that time. First, *B. neotomae* was able to ferment a broader range of carbohydrates compared to *B. abortus*, *B. melitensis* and *B. suis* and produced acid without gas in dextrose, levulose, xylose, arabinose and galactose (Stoenner and Lackman 1957). Second, these rodent strains also showed evident differences in oxidative rates on certain amino acid substrates (Cameron and Meyer 1958). Third, they were sensitive to both thionine and basic fuchsin. Finally, *B. neotomae* proved to be more infective for mice than for guinea pigs (Stoenner and Lackman 1957).

Experimental infection of swine with *B. neotomae* resulted in bacteremia (5–7 weeks) without apparent clinical manifestation (Beal et al. 1959). Though a parent strain of *B. neotomae* was not fatal for Swiss Webster mice, one of the sub-strains derived in serial transfers became significantly more virulent. This strain was 100% lethal at all doses, even at a dose as low as 2000 bacterial cells (Gibby and Gibby 1965).

B. neotomae has been considered a non-zoonotic bacterium for 60 years but was recently implicated as a potential human pathogen after being isolated from the cerebrospinal fluid of two independent cases with neurobrucellosis in Costa Rica (Suárez-Esquivel et al. 2017; Villalobos-Vindas et al. 2017). *B. neotomae* was retrospectively identified by whole-genome sequencing and phylogenetic analysis. Using traditional microbiological methods *B. abortus*, the major cause of brucellosis in Costa Rica, can only be differentiated by its oxidase activity. Further investigations in murine and cellular models of infection revealed pathogenic attributes of *B. neotomae* comparable to zoonotic *Brucella* spp. (Kang and Kirby 2017; Kang, Brown, and Kirby 2019; Waldrop and Sriranganathan 2019). These reports suggested that some cases of human brucellosis were, in fact, misdiagnosed cases of *B. neotomae* (Suárez-Esquivel et al. 2017). However, recent extensive whole-genome sequence analysis of *B. neotomae* strains revealed an unexpected identical genotype both in the two Costa Rican strains (without known epidemiological linkage), and with one of the strains originally isolated in Utah in the 1950s which calls for further sampling to better understand the epidemiology and zoonotic potential of *B. neotomae* (Vergnaud et al. 2024).

4 | Novel *Brucella* spp.

In North Queensland, Australia, seven *Brucella* strains, initially identified as *B. suis* bv 3, were isolated from three different native rodent species in 1964 (Cook, Campbell, and Barrow 1966). These rodent strains were genetically identical to each other and had a unique 16S rRNA sequence. Multi-locus sequence typing of *rpoB*, *recA* and nine other genes revealed a taxonomic position distant from the core *Brucella* clade (Tiller et al. 2010a). Comparative genomics proved similarities between the Australian rodent strains and *B. inopinata* BO1 and BO2 human isolates (Wattam et al. 2012). Furthermore, these strains were phenotypically different from all other *Brucella* spp., including the rodent species *B. neotomae* and *B. microti* (Tiller et al. 2010a).

4.1 | *Brucella vulpis*

A molecular survey of *Brucella* in rodents and shrews collected throughout Germany showed a high prevalence in this natural reservoir (Hammerl et al. 2017). Most of the positive animals were bank voles (*Myodes glareolus*) and mice of the genus *Apodemus*. *recA* typing demonstrated that these rodent-borne brucellae were phylogenetically closely related to a novel *Brucella* species, *B. vulpis*, initially isolated from mandibular lymph nodes of red foxes in Austria (Hofer et al. 2012).

4.2 | *Brucella microti*

A novel *Brucella* species was first isolated from common voles (*Microtus arvalis*) during an outbreak of epizootic brucellosis in a vole population in southern Moravia (Czech Republic) between 1999 and 2003 (Hubálek et al. 2007). Rigorous genetic and phenotypic investigations demonstrated that the isolates obtained from voles were clearly different from all hitherto known *Brucella* species. According to the primary host, the novel species was named *Brucella microti* (Scholz et al. 2008b).

Compared to the six classical *Brucella* spp. and other *Brucella* species, which reveal low metabolic activity and are considered fastidious and fully adapted to intracellular growth, *B. microti* can easily grow on standard media such as tryptic soy or meat peptone agar without supplementary CO_2. In addition, *B. microti* exhibits striking metabolic characteristics which are common in soil bacteria such as *Ochrobactrum* spp. (Occhialini et al. 2022). The extraordinary metabolic activity of *B. microti* may lead to confusing test results when traditional microbiological methods such as API20NE are applied because they usually misidentify *B. microti* as *Ochrobactrum anthropi* (Hubálek et al. 2007; Scholz et al. 2008b). Some researchers hypothesize that *B. microti* and other atypical *Brucella* spp. (*B. inopinata*, *B. inopinata*- and *B. microti*-like strains) represent an older lineage and may be the intermediate stage between free-living environmental *Ochrobactrum* and the facultative intracellular zoonotic pathogens such as *B. melitensis* (Al Dahouk et al. 2012; Audic et al. 2009; Morris Jr. and Southwick 2010; Scholz et al. 2016a).

Subsequently, *B. microti* was isolated from soil in the Czech Republic and also from the mandibular lymph nodes of red fox (*Vulpes vulpes*) in Austria (Scholz et al. 2008a, 2016b) and wild boar (*Sus scrofa*) in Hungary (Rónai et al. 2015). Most recently, *B. microti*-like strains were found in the native moor frog (*Pelophylax ridibundus*) originating from a French farm (Jaÿ et al. 2018, 2020).

5 | Pathogenic Properties of *Brucella microti*

During the first epizootic event in the Czech Republic, remarkable clinical signs were observed in the voles naturally infected with *B. microti*, such as edematous extremities, arthritis, lymphadenitis, skin perforation due to abscesses, orchitis or peritoneal granulomas (Hubálek et al. 2007). Experimental infection of ICR mice resulted in the death of 50% of the animals (Hubálek et al. 2007). Both the *virB* operon and the *wbkE* gene involved in O-polysaccharide synthesis play a crucial role in the lethality of *B. microti* in the murine model of infection as demonstrated by Hanna et al. (2011) and Ouahrani-Bettache et al. (2019), respectively. In another experimental model, 10^5 colony-forming units of *B. microti* killed 82% of BALB/c mice within 7 days (Jiménez de Bagüés et al. 2010). These observations suggest that *B. microti* is a highly pathogenic bacterium for common voles and other rodents (Hubálek et al. 2007). Because of the high mortality rate and the lack of a specific life cycle in voles, *B. microti* is probably a soil bacterium that only occasionally infects rodents (and other mammals) through the ingestion of contaminated products (e.g., feed). The transmission among rodents may be possible by direct contact such as biting and scratching.

Further studies of in vitro and in vivo pathogenesis revealed that *B. microti* replicates not only in murine macrophage-like J774 cells but also in human macrophage-like THP-1 cells and human monocyte-derived macrophages at higher rates than *B. suis* bv 1 strain 1330. One of the possible explanations for the high replication rate is the higher resistance of *B. microti* to acidic pH compared with *B. suis* (Jiménez de Bagüés et al. 2010). Occhialini et al. (2022) demonstrated the role of the glutamic acid decarboxylase system (GAD) in the acid resistance of *B. microti*, while the six classical *Brucella* species are GAD negative and are thus less resistant to acidic stress (Damiano et al. 2015). Experiments comparing infection in wild-type mice, Jh mice (lacking B cells), SCID mice (lacking T and B cells), and SCID Beige mice (lacking T, B, and NK cells) showed that both B and T cells are important for controlling infection. NK cells are the key to survival in the absence of B and T cells (Jiménez de Bagüés et al. 2011).

Since a possible case of human infection with *B. microti* was recently reported in the Czech Republic, the human pathogenicity of this species has to be reassessed (Hubálek et al. 2023). A zoologist was bitten in her finger by a vole caught in a live trap showing typical signs of the disease including colliquated abscesses (Hubálek et al. 2007). The animal was euthanized on the same day, and *B. microti* was isolated from liver and kidneys (Hubálek et al. 2007). Subsequently, the patient developed fever (39.5°C), chills, general weakness, headaches, joint and back pain. A small ulcer of the injured finger was accompanied by enlarged and moderately painful axillary lymph nodes (3–4 cm) on the same side. Lymphadenopathy was also found in the inguinal region as well as foot and ankle oedema. In-house *B. microti* antigen preparations agglutinated moderately in the SAT (serum agglutination test) at a serum dilution of 1:80 two months after the bite (Hubálek et al. 2023).

6 | Detection of *B. microti* in Non-rodent Species

In the case of *B. microti*, a saprophytic lifestyle (bacteria that live on, and get their nourishment from, dead organisms or decaying organic material) should also be considered, because fully viable bacteria were isolated from soil samples 6 months after the vole epizootic. Hence, *B. microti* is the only member of the genus *Brucella* (except for the recently included *Ochrobactrum* spp.) whose presumed reservoir might be outside of an animal species and brucellosis could be a sapronosis (infectious disease directly transmitted from an abiotic environment such as soil) rather than a zoonosis in this case (Scholz et al. 2008a). Isolation of *B. microti* from (sub)mandibular lymph nodes of red foxes in Austria and wild boars in Hungary may also suggest environmental transmission cycles of *B. microti*. Neither red foxes nor wild boars showed relevant pathology, thus likely representing healthy carriers of *B. microti*. The red foxes may have acquired the bacterium by hunting and eating infected rodents, and the wild boars by ingesting rodent carcasses, or burrowing in soil (Rónai et al. 2015; Scholz et al. 2016b). Given the detection of *B. microti* in rodents and soil, and the occurrence of *B. microti*-like strains in farmed frogs, awareness of the potential public health risk should be raised, especially among highly exposed groups such as farmers, hunters, forestry workers, zoologists, veterinarians as well as laboratory technicians (Ackelsberg et al. 2020; Jaÿ et al. 2018; Jaÿ et al. 2020; Scholz et al. 2008a; Scholz et al. 2008b). The possible routes of spread of *B. microti* in the environment are shown in Figure 1.

7 | Conclusions and Perspectives

The most common transmission routes of the major *B. melitensis* and *B. abortus* species from livestock to humans, via contaminated dairy products or direct contact with infected livestock and their reproductive material, seem unlikely in the case of rodent brucellosis. Considering our increasing awareness of the pathogenic potential of *B. microti*, it is still quite surprising that human cases have not been confirmed until recently. Some patients actually infected with rodent-borne *Brucella* may have been misdiagnosed as *Ochrobactrum anthropi* or *O. intermedium* infections (due to the well-known misidentification by standard microbiological methods). In addition, the vast majority of brucellosis cases are diagnosed serologically and the standard serological tests cannot differentiate between smooth *Brucella* species. Only additional selective culture methods or molecular tests, rarely applied routinely, allow for further differentiation to the species level. Hence, there is always a great chance that human brucellosis cases do occur but go unnoticed (Pappas 2010). Furthermore, brucellosis is undoubtedly one of the most misdiagnosed diseases, because it presents with a

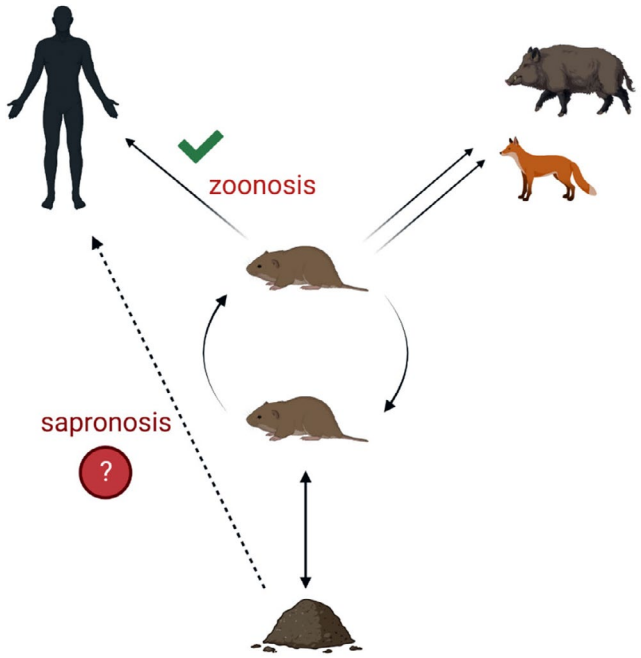

FIGURE 1 | Potential transmission routes of *B. microti* in Central Europe. Black arrows represent highly probable routes, whereas the dashed arrow shows a possible route of transmission (yet to be fully confirmed).

variety of clinical signs and symptoms and often non-specific and atypical manifestations (Pappas 2010; Zheng et al. 2018). The reasons for brucellosis misdiagnosis are not only related to clinical manifestations but also to other factors such as the transient presence of the bacterium in blood and slow growth of most *Brucella* strains impacting culture sensitivity as well as the poor sensitivity and specificity of serological tests in chronic infections. Therefore, a public health risk of rodent-borne brucellosis seems to be plausible (Al Dahouk et al. 2012; González-Espinoza et al. 2021; Jiménez de Bagüés et al. 2010; Morris Jr. and Southwick 2010).

The recent discovery of novel and phenotypically different *Brucella* strains and an increasing number of human cases caused by them should initiate targeted monitoring and comprehensive surveillance in wildlife and the environment. As serologic diagnosis may be problematic in brucellosis caused by the non-core species, which have atypical and diverse LPS structures (Al Dahouk et al. 2017; Soler-Lloréns et al. 2016; Wattam et al. 2012; Zygmunt et al. 2012), and thus may not be detected by existing serological tests targeting LPS, surveillance based on bacteriological or molecular approaches is likely to be more useful. To assess the prevalence of *B. microti* and its public health risk, we propose (1) comprehensive screening of rodents and other wildlife mammals (e.g., bats, badgers, foxes, wild boars) as well as environmental samples (mainly soil) using molecular techniques and culture methods, and (2) the evaluation of rodent *Brucella* spp. as potential etiologic agents of febrile illness with non-specific signs and symptoms in individuals belonging to high-risk groups (farmers, hunters, veterinarians, zoologists). Based on such a screening, we will gain more comprehensive knowledge about natural hosts and routes of transmission of *B. microti* in the environment and identify possible sources of human infection (Mühldorfer et al. 2017; Whatmore and Foster 2021). According to the Czech National Institute of Public Health, bovine and caprine brucellosis have been eradicated in the Czech Republic for decades, while *B. suis* biovar 2 is widespread among pigs and hares (Kolbabová, Havlasová, and Veleba 2001). Cases of human brucellosis are rarely reported in the Czech Republic and are always imported from endemic countries (Mand'áková 2024). In 2021, 165 confirmed brucellosis cases were reported in the EU/EEA. The notification rate in the EU/EEA was 0.04 cases per 100,000 population. The highest number of cases were reported in Italy, Spain, Greece and France (ECDC 2023). Against this epidemiological background, serological screening in subpopulations with a high exposure risk could provide further insights into the zoonotic potential of *B. microti*. Initial seroprevalence studies should include professionals at risk of an infection with *B. microti*, such as farmers who are in close contact with potentially contaminated soil and rodents nearby their farms. Hunters and veterinarians might also be exposed with a higher probability since *B. microti* has been detected in wildlife animals such as feral pigs and foxes (Hammerl et al. 2017; Rónai et al. 2015; Scholz et al. 2016b, 2008a).

In recent years, it has become apparent that we are facing the emergence of brucellosis worldwide. Not only classical *Brucella* species are re-emerging, but also novel species are increasingly associated with human disease. *B. microti* is one of the novel species that is forcing us to re-evaluate the impact of *Brucella* on public health. Further research on the ecological persistence of rodent-borne *Brucella* in the environment, their epizootic role in wild rodent populations, and their virulence and pathogenicity in other mammals including humans is urgently needed.

Author Contributions

Conceptualization: Ivo Rudolf, Michael Kosoy, Sascha Al Dahouk. Supervision: Sascha Al Dahouk. Visualization: Ivo Rudolf with BioRender software. Writing – original draft: Ivo Rudolf, Romana Kejíková, Micheal Kosoy, Sascha Al Dahouk. Writing – review and editing: Ivo Rudolf, Silvie Šikutová, Kristína Mravcová, Zdeněk Hubálek, Sascha Al Dahouk, Adrian M. Whatmore.

Acknowledgements

Open access publishing facilitated by Ustav biologie obratlovcu Akademie ved Ceske republiky, as part of the Wiley – CzechELib agreement.

Ethics Statement

The authors state that ethical approval is not required. Neither animal nor human studies were conducted.

Consent

Written informed consent for publication was obtained from all authors of the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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