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SHORT COMMUNICATION

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

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

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

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

[3]Naturhistorisches Museum Wien, Vienna, Austria

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

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

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

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

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

[9]Swiss Institute of Bioinformatics, Lausanne, Switzerland

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

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Abstract

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

KEYWORDS

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

1 | INTRODUCTION

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

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

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

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

2 | MATERIALS AND METHODS

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

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

The serological detection of DOBV-reactive antibodies in chest cavity fluid samples of mice used an established IgG ELISA with a recombinant DOBV nucleocapsid protein as antigen (see Appendix S1).

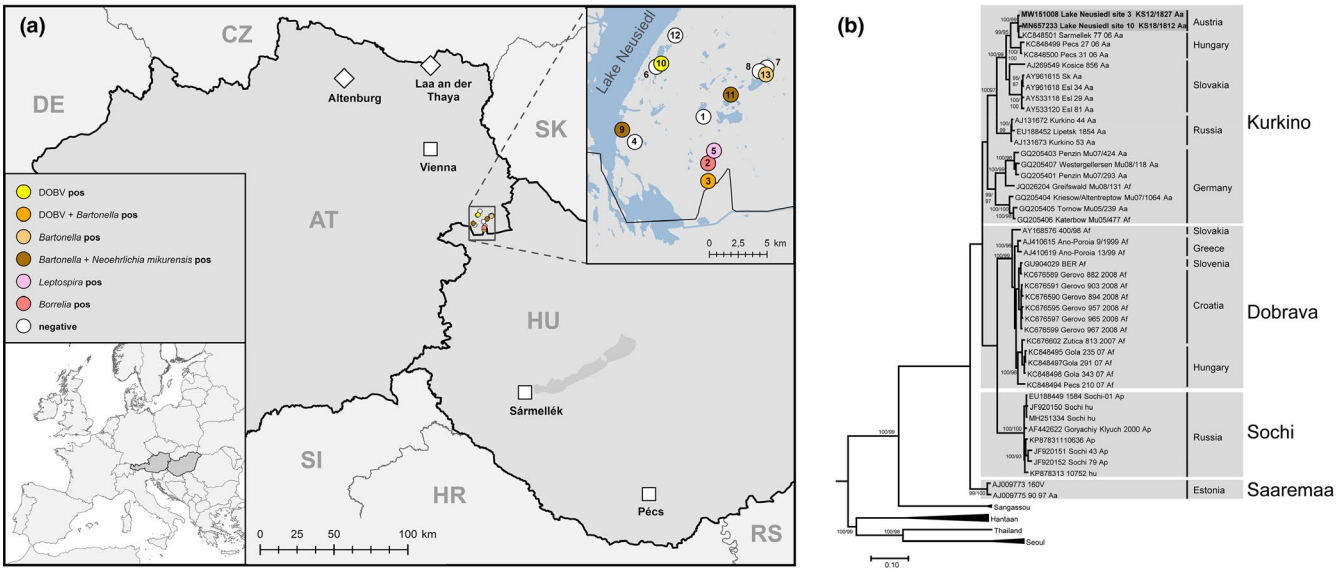

FIGURE 1 Trapping sites in Austria with two inserts showing the location in Europe and the magnified area with numbered sites (a) and consensus phylogenetic tree of Dobrava–Belgrade orthohantavirus (DOBV) based on partial nucleocapsid protein-coding S segment sequences (b). Trapping sites where previously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

3 | RESULTS

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

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

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

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

4 | DISCUSSION

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

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

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

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

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

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

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

DATA AVAILABILITY STATEMENT

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

ORCID

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

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SUPPORTING INFORMATION

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

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