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Multiple Infections of Rodents with Zoonotic Pathogens in Austria

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Abstract

Rodents are important reservoirs for a large number of zoonotic pathogens. We examined the occurrence of 11 viral, bacterial, and parasitic agents in rodent populations in Austria, including three different hantaviruses, lymphocytic choriomeningitis virus, orthopox virus, *Leptospira* spp., *Borrelia* spp., *Rickettsia* spp., *Bartonella* spp., *Coxiella burnetii*, and *Toxoplasma gondii*. In 2008, 110 rodents of four species (40 *Clethrionomys glareolus*, 29 *Apodemus flavicollis*, 26 *Apodemus sylvaticus*, and 15 *Microtus arvalis*) were trapped at two rural sites in Lower Austria. Chest cavity fluid and samples of lung, spleen, kidney, liver, brain, and ear pinna skin were collected. We screened selected tissue samples for hantaviruses, lymphocytic choriomeningitis virus, orthopox viruses, *Leptospira*, *Borrelia*, *Rickettsia*, *Bartonella* spp., *C. burnetii*, and *T. gondii* by RT-PCR/PCR and detected nucleic acids of Tula hantavirus, *Leptospira* spp., *Borrelia afzelii*, *Rickettsia* spp., and different *Bartonella* species. Serological investigations were performed for hantaviruses, lymphocytic choriomeningitis virus, orthopox viruses, and *Rickettsia* spp. Here, Dobrava-Belgrade hantavirus-, Tula hantavirus-, lymphocytic choriomeningitis virus-, orthopox virus-, and rickettsia-specific antibodies were demonstrated. Puumala hantavirus, C. *burnetii*, and *T. gondii* were neither detected by RT-PCR/PCR nor by serological methods. In addition, multiple infections with up to three pathogens were shown in nine animals of three rodent species from different trapping sites. In conclusion, these results show that rodents in Austria may host multiple zoonotic pathogens. Our observation raises important questions regarding the interactions of different pathogens in the host, the countermeasures of the host's immune system, the impact of the host–pathogen interaction on the fitness of the host, and the spread of infectious agents among wild rodents and from those to other animals or humans.

Key Words: Rodents—Rodent-borne pathogens—Tick-borne pathogens—Austria—Multiple infections.

Introduction

In the last decades, the incidence of human diseases caused by zoonotic viruses, bacteria, and parasites that are associated with small mammal reservoirs appears to have increased (Meerburg et al. 2009). In Europe bank vole-associated Puumala virus (PUUV), different genotypes of Dobrava-Belgrade virus (DOBV) hosted by various *Apodemus* species and perhaps Tula virus (TULV) cause hemorrhagic fever with renal syndrome (HFRS) of different severity

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(Heyman et al. 2011, Klempa et al. 2013). For some other viral agents, such as lymphocytic choriomeningitis virus (LCMV) and cowpox virus (CPXV), a member of the genus *Orthopoxvirus* (OPV), the role of rodent reservoirs in Central Europe is unknown. LCMV causes infections in humans of varying severity from asymptomatic disease to severe meningitis (Emonet et al. 2007, Ceianu et al. 2008, Pérez-Ruiz et al. 2012), and sporadic CPXV infections have been described in humans, domestic, and zoo animals (Essbauer et al. 2010).

For bacterially induced zoonoses, leptospirosis is an emerging disease of global importance with a variation in the severity of symptoms (Bharti et al. 2003). Outbreaks are often associated with agricultural work or leisure activities involving exposure to freshwater (Desai et al. 2009). *Bartonella henselae* is the most important pathogenic *Bartonella* species in Europe. It is transmitted by cats and causes cat scratch disease and more rarely endocarditis, bacillary angiomatosis, and peliosis hepatis in immunodeficient patients (Kaiser et al. 2011). For many *Bartonella* spp., the pathogenicity is not known (*e.g*., *B. taylorii, B. doshiae, B. birtlesii*), but some have been proven to cause endocarditis, bacterimia, and neuroretinitis (*B. grahamii*, *B. tamiae*) (Breitschwerdt et al. 2013). *Coxiella burnetii* may cause severe infections, *i.e*., Q fever with pneumonia as typical symptom. The main sources for these infections are infected ruminants in which the agent may cause abortion and infertility. But other mammals, including rodents, are susceptible to infection with *C. burnetii* and may contribute to its transmission (Meerburg and Reusken 2011).

Rodents are also considered important reservoirs for different arthropod-borne bacteria (Hoogstraal 1967, Stanek and Strle 2003). *Borrelia afzelii*, the most prevalent spirochete causing Lyme disease in Europe, is perpetuated in a cycle involving rodents and *Ixodes ricinus* ticks (Richter et al. 2004a). *Borrelia bavariensis, B. spielmanii*, and *B. burgdorferi* sensu stricto (s.s.) are also associated with rodents, but generally infect fewer questing ticks (Richter et al. 2004b, Margos et al. 2009). Rickettsiosis is an increasing health problem in Europe (Parola and Raoult 2001), but studies in rodents as reservoirs are rare (Spitalská et al. 2008). Recently, a rodent survey identified *Rickettsia felis* and *R. helvetica* in rodents in southeastern Germany (Schex et al. 2011).

Rodents are also involved in the transmission cycles of endoparasites, such as *Toxoplasma gondii* (Mills and Childs 1998). Ingestion of *T. gondii*–infected tissues by felids, *e.g*., domestic cats, may result in shedding of high numbers of environmentally resistant oocysts, from which infection is passed orally to humans (Dubey et al. 2004). Prenatal infections may cause abortion, and postnatal infections of immune-suppressed persons cause serious and occasionally fatal illness.

Here, we describe a survey for selected viruses, bacteria, and parasites in rodents captured in two areas in Lower Austria.

Materials and Methods

Rodent trapping and necropsy

In October, 2008, rodents were trapped in 565 snap traps during one night at five rural sites in the municipality of Laa an der Thaya and two rural sites in the municipality of Altenburg, northern Lower Austria, near the Czech border (Table 1, Fig. 1). Rodent necropsy and collection of chest cavity fluid (CCF) and tissue samples followed previously established standard protocols. Morphological species determination was confirmed by PCR and sequencing of the partial mitochondrial *cytochrome b* (*cyt b*) gene (Fink et al. 2010, Schlegel, et al. 2012b). Rodent species and genetic affiliations within species were determined by sequence comparisons against GenBank entries using the BLAST algorithm (www.ncbi.nlm.nih.gov) and against species-specific *cyt b* datasets covering all genetic lineages within these rodents (Michaux et al. 2003, Heckel et al. 2005, Michaux et al. 2005, Dubey et al. 2009, Wójcik et al. 2010, Sutter et al. 2013).

Serology

Serological investigations of CCF samples were performed using previously published protocols (Table 2).

Nucleic acid isolation

DNA and RNA were extracted from tissue samples using commercial kits (Qiagen Tissue Kit, QIAamp DNA Mini Kit, Qiagen, Hilden, Germany; Nucleospin DNA Tissue Kit, Macherey-Nagel, Düren, Germany; RTP DNA/RNA Virus Mini Kit, Invitek, Berlin, Germany) according to the manufacturers' instructions. Alternatively, RNA extraction was performed using a modified QIAzol extraction protocol (Schlegel et al. 2012a).

RT-PCR, PCR, and sequence analysis

Various published real-time and conventional RT-PCR/PCR and standard sequencing protocols were used for screening for viral, bacterial, and parasite-derived nucleic acids (Table 3). In addition, a conventional *Toxoplasma*-specific PCR and a novel *Bartonella*-specific real-time PCR targeting a fragment of the β-subunit of bacterial RNA polymerase were performed (for details, see Table 3).

Results

Rodent trapping

A total of 110 rodents were captured including 29 *Apodemus flavicollis*, 26 *A. sylvaticus*, 40 *Clethrionomys glareolus* (for the valid generic name of the bank vole, see Tesakov et al. 2010), and 15 *Microtus arvalis* (Table 1). The capture of 19.5 rodents consisting of only four species per 100 trap nights indicates a very high abundance of relatively low rodent diversity. According to the *cyt b* sequences, all rodents belonged to a single genetic lineage per species, each with large geographic distribution. Bank voles belonged to the Carpathian lineage (distribution, Eastern Europe/Balkans; Wojcik et al. 2010) and all common voles to the Eastern lineage (Eastern Europe; Heckel et al. 2005). Yellow-necked field mice and wood mice were represented by the clade C (Western Palaearctic distribution; Michaux et al. 2005) and the subclade 2b (Western/Central/Northern Europe; Michaux et al. 2003), respectively.

Detection of viral infections

Serological screening of bank voles for hantavirus (PUUV)-specific antibodies and PUUV/TULV S-specific RT-PCR revealed no positive animal (Table 1). One of the 29 (3.4%) yellow-necked field mice was seropositive in the DOBV-immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) (Table 1), whereas none of the wood

Table 1. Description of Trapping Sites, Rodents Trapped, and Pathogens Found

Trapping locality	*Site*	*Vegetation*	*Species*	*No. of trapped animals*	*Pathogen detection (no. of positive animals/total no. of animals analyzed)*	
					PCR or RT-PCR	*Serology*
Altenburg[a]	A1	Tall forbs, thick grass layer, bordering to a field	*Apodemus flavicollis*	4	*Leptospira* spp. (3/4), *Borrelia afzelii* (1/4)	*Rickettsia* spp. (1/4)
			Apodemus sylvaticus	1	None	*Rickettsia* spp. (1/1)
	A2	Bank slope with a thick grass and herbal layer	*Apodemus flavicollis*	1	None	None
			Apodemus sylvaticus	3	*Leptospira* spp. (2/3)	None
Laa an der Thaya[b]	L1	Overgrown sand pit with grassy ground, tall forbs, elder, and robinia bushery	*Apodemus flavicollis*	2	None	None
			Apodemus sylvaticus	4	*Borrelia afzelii* (1/4)	None
			Clethrionomys glareolus	8	*Borrelia afzelii* (1/8)	*Rickettsia* spp. (1/8)
			Microtus arvalis	7	TULV (1/7), *Leptospira* spp. (1/7), *Borrelia afzelii* (4/7), *Rickettsia* spp.(2/7), *Bartonella taylorii* (2/7)	TULV (1/7), *Rickettsia* spp. (1/7)
	L2	Edge of a robinia–ash forest, bordering to a field	*Apodemus flavicollis*	10	*Borrelia afzelii* (1/10), *Bartonella taylorii* (1/10)	DOBV (1/10)
			Apodemus sylvaticus	5	*Bartonella taylorii* (1/5), *Bartonella grahamii* (2/5)	LCMV (1/5)[c]
			Clethrionomys glareolus	5	None	*Rickettsia* spp. (1/5)
			Microtus arvalis	4	TULV (1/4), *Borrelia afzelii* (2/4)	TULV (1/4), *Rickettsia* spp. (1/4)
	L3	Base of an embankment at the Thaya with a thick hedge of robinia, blackthorn, ash, and *Euonymus europaeus*	*Apodemus flavicollis*	4	None	*Rickettsia* spp. (1/4)
			Apodemus sylvaticus	8	*Bartonella birtlesii* (1/8), *Bartonella taylorii* (2/8)	None
			Clethrionomys glareolus	5	*Leptospira* spp. (2/5), *Borrelia afzelii* (1/5)	None
			Microtus arvalis	4	*Borrelia afzelii* (2/4), *Borrelia garinii* (1/4)	*Rickettsia* spp. (1/4)
	L4	Robinia and ash forest with grassy ground and local stinging-nettle populations	*Apodemus flavicollis*	4	*Borrelia afzelii* (1/4), *Bartonella taylorii* (1/4)	None
			Clethrionomys glareolus	11	*Leptospira* spp. (1/10[d]), *Borrelia afzelii* (1/11), *Rickettsia* spp. (1/10[d])	*Rickettsia* spp. (1/11), OPV (1/7[d])[c]
	L5	Northern bank of the Thaya with extended goldenrod vegetation and moist ground	*Apodemus flavicollis*	4	*Bartonella taylorii* (1/3[d])	None
			Apodemus sylvaticus	5	*Bartonella taylorii* (1/5)	*Rickettsia* spp. (1/5)
			Clethrionomys glareolus	11	*Bartonella doshiae* (1/11)	*Rickettsia* spp. (1/11)

[a]A1: 65 traps at WGS 84: 48.63086 N 15.61078 E 281 m, and A2: 135 traps at WGS84: 48.62995 N 15.61493 E 266 m.
[b]L1: 65 traps at WGS 84: 48.73685 N 16.33875 E 182 m; L2: 70 traps at WGS 84: 48.73583 N 16.33818 E 184 m; L3: 100 traps at WGS 84: 48.73587 N 16.34259 E 183 m; L4: 50 traps at WGS 84: 48.73450 N 16.34060 E 178 m; L5: 80 traps at WGS 84: 48.73571 N 16.34543 E 182 m.
[c]The Pan-arenavirus RT-PCR and the OPV-PCR were negative.
[d]For this analysis samples were not available for all animals.
TULV, Tula virus; DOBV, Dobrava-Belgrade virus; OPV, orthopox virus; LCMV, lymphocytic choriomeningitis virus.

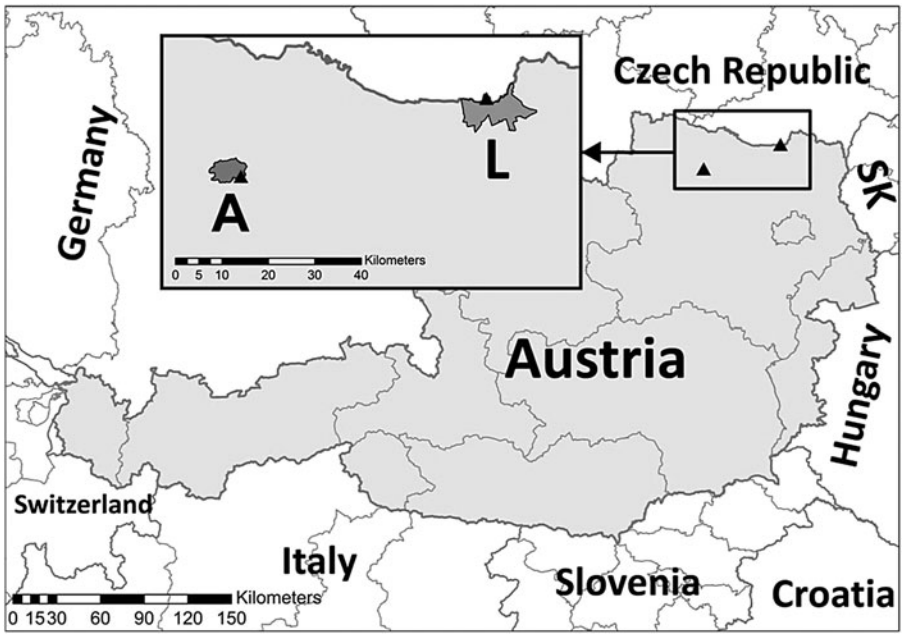

FIG. 1. Map of trapping sites at the municipality Laa an der Thaya (L) and the municipality Altenburg (A) in Lower Austria. SK, Slovakia.

mice contained DOBV-specific antibodies. Hantavirus RNA was not detected in any of the investigated *Apodemus*-derived lung samples. TULV was the only hantavirus detected by serological and molecular methods in two of the 15 (13.3%) common voles (Table 1). A phylogenetic analysis of the obtained S segment sequences (accession nos. KF184327 and KF184328) demonstrated their close relationship to previously published TULV sequences from Austria (similarity of 92–98%) and Slovakia and Czech Republic (94–97%; Bowen et al. 1997; data not shown). LCMV-specific antibodies were detected in one of 26 (3.8%) wood mice, but not in any other species. Subsequent Pan-arenavirus RT-PCR analysis did not amplify any LCMV-specific RNA (Table 1). One CCF sample of 29 analyzed bank voles produced a weak signal in the OPV-IFA, but OPV-DNA was not detected by PCR in any rodent (Table 1).

Detection of bacterial and T. gondii *infections*

For the bacteria, the *lipl32 Leptospira*-PCR assay revealed a specific product for eight of 109 (7.3%) examined kidney samples (Table 1). The duplex PCR identified *L. kirschneri* in two wood mice and one yellow-necked field mouse from Altenburg, whereas products indicating infection with the genomospecies *L. interrogans*, *L. borgpetersenii*, *L. weilii*, *L. noguchii*, *L. santarosai*, or *L. meyeri* were amplified from two bank voles and one common vole from Laa an der Thaya. Of the remaining two *lipl32*-PCR positive rodents, one was negative (bank vole) in the duplex PCR approach; the other (yellow-necked field mouse) could not be further analyzed by this assay.

Borrelia-specific DNA was detected by nested PCR in a total of 16 animals (14.8%) of all four examined species, with the highest prevalence (53.3%) in common voles (Table 1). Subsequent sequencing confirmed *B. afzelii*–specific DNA for 15 samples; for one common vole, a co-infection by *B. afzelii* and *B. garinii* was found (Table 4).

Indirect IFA investigation using *Rickettsia conorii* as the spotted-fever group (SFG) antigen demonstrated reactivity in 11 animals of all four species and both trapping sites, most frequently in *M. arvalis* ($n = 2/15$; 13.3%) (Table 1). Pan-rickettsial PCR analysis revealed three positive tissue samples (Table 1). The amplification of the *omp*B fragment

Table 2. Overview of the Serological Methods Used for Screening Rodents for Zoonotic Agents

Pathogen	*Method*	*Reference*
Puumala virus	ELISA	Mertens et al. 2011
Dobrava–Belgrade virus	ELISA	Schlegel et al. 2009
Tula virus	ELISA	Schlegel et al. 2012a
Lymphocytic choriomeningitis virus	Indirect Immunofluorescence	Ceianu et al. 2008, Coulybaly-N'Golo et al. 2011
Orthopox virus[a]	Indirect Immunofluorescence	Appl et al. 2013
Rickettsia spp.	Indirect Immunofluorescence	*Rickettsia conorii* Panbio IF Kit; for details see Schex et al. 2011

[a]Due to the cross-reactivity of orthopox viruses, this assay detects also cowpox virus–specific antibodies.
ELISA, enzyme-linked immunosorbent assay.

Table 3. Overview of the Molecular Methods Used for Screening Rodent Samples for Zoonotic Agents

Pathogen	*Tissue*	*Method*	*Target*	*Reference*
Puumala virus	Lung	Conventional RT-PCR	Partial S segment (760 bp)	Essbauer et al. 2006
Dobrava–Belgrade virus	Lung	Conventional RT-PCR	Partial L segment	Klempa et al. 2006
Tula virus	Lung	Conventional RT-PCR and direct sequencing	Partial S segment (760 bp)	Essbauer et al. 2006
Lymphocytic choriomeningitis virus	Spleen	Conventional RT-PCR	Partial Lassavirus L gene	Coulybaly-N'Golo et al. 2011, Vieth et al. 2007
Orthopox virus (OPV)[a]	Liver	Real-time PCR	Partial hemagglutinin gene	Qiagen-Artus Orthopox LC PCR Kit; Olson et al. 2004
Leptospira spp.	Kidney	Conventional PCR	Partial *flaB* (563-bp fragment)	Gravekamp et al.1993
			Partial *secY* (285-bp fragment)	Bal et al. 1994, Levett et al. 2005
			Partial *lipl* (423-bp fragment)	Haake et al. 2000, Mayer-Scholl et al. 2011
Borrelia spp.	Skin	Nested conventional PCR and direct sequencing	Partial 16S rRNA (600 bp)	Richter et al. 2006, 2013
Rickettsia spp.	Skin	Screening real-time PCR	Partial *gltA*	Wölfel et al. 2006,
		Conventional PCR	Partial *ompB*	Schex et al. 2011
Bartonella spp.	Spleen	Real-time screening PCR	Partial *rpoB* (78 bp)	This paper[b]
		Conventional confirmatory PCR and direct sequencing	ITS (419–565 bp)	Maggi and Breitschwerdt 2005
Coxiella burnetii	Liver	Screening real-time PCR	Partial *IS1111*	Schrader et al. 2000
		Nested conventional PCR	Partial *com1*	Zhang et al. 1998
Toxoplasma gondii	Brain	Conventional PCR	529-bp repeat	Reischl et al. 2003, Homan et al. 2000, this paper[c]

[a]Detects OPV including also cowpox virus (CPXV).
[b]With QuantiFast Probe PCR kit (Qiagen) according to the manufacturers' protocol using primers BART F1(5′-AGA AGA GTT TGT TGT TTG CC), BART F2 (5′-AGA AGA GTT TGT TGT TTG TC), BART R (5′-GAA ACA TCC ATC AAA TCA ACA TG) and LNA probe BART-P (5′-FAM- AAA CTT CAC CAG CAT GA-BHQ1.
[c]Primers TOX-8 (0.5 μM) in combination with Tox5 (0.5 μM) were used with the Dynazyme II F-501L polymerase (Finzyme, Espoo, Finland). Cycling was performed at 94°C for 1 min, followed by 35 cycles of 60°C for 1 min, 72°C for 1 min, and 94°C for 1 min, and a final extension at 72°C for 10 min.
FAM, 6-carboxyfluorescein; BHQ1, black hole quencher 1.

was not possible, and thus the species could not be characterized.

Initial real-time PCR analysis for *Bartonella* produced a total of 21 positive samples, but only 12 samples were confirmed by conventional PCR (Table 1). Subsequent sequencing identified *B. taylorii* in three yellow-necked field mice, three wood mice, and two common voles. *B. grahamii* was exclusively found in two wood mice, *B. doshiae* in one bank vole, and *B. birtlesii* in one wood mouse.

Table 4. Detection of Multiple Infections in Austrian Rodents

Species (frequency of multiple infection)	*Trapping site*	*Pathogens*[a]
Apodemus flavicollis (2/29)	Altenburg, site 1	*Borrelia afzelii* and *Leptospira* spp.
	Laa an der Thaya, site 4	*Borrelia afzelii* and *Bartonella taylorii*
Clethrionomys glareolus (1/40)	Laa an der Thaya, site 1	*Borrelia afzelii* and *Rickettsia* spp. (serology)
Microtus arvalis (6/15)	Laa an der Thaya, site 1	*Borrelia afzelii*, *Rickettsia* spp. (PCR)
	Laa an der Thaya, site 1	*Borrelia afzelii*, *Bartonella taylorii* and *Rickettsia* spp. (serology)
	Laa an der Thaya, site 1	*Borrelia afzelii,* Tula virus (RT-PCR, serology), *Bartonella taylorii*
	Laa an der Thaya, site 2	*Borrelia afzelii* and *Rickettsia* spp. (serology)
	Laa an der Thaya, site 2	*Borrelia afzelii,* Tula virus (RT-PCR, serology)
	Laa an der Thaya, site 3	*Borrelia afzelii, Borrelia garinii*

[a]Detection method is given in brackets for the aforementioned pathogen, when RT-PCR/PCR and a serological method were used.

C. burnetii and *T. gondii* infections were not detected in any of the animals.

Multiple infections

Double and triple infections were detected in seven and two of 110 (6.4% and 1.8%) rodents respectively, comprising three of four rodent species (Table 4). Common voles were most frequently infected by more than one pathogen. *B. afzelii* was detected in all multiply infected animals. Three multiply infected animals harbored *B. taylorii*. Co-infections with *Rickettsia* spp. were demonstrated in three of four animals only by serology. Both common voles harboring TULV RNA also contained DNA of *B. afzelii* and one additionally DNA of *B. taylorii*.

Discussion

This molecular and serological survey of 110 rodents from Lower Austria demonstrated 50 animals being infected by at least one pathogen, including hantaviruses (TULV and DOBV), LCMV, OPV, *Leptospira* spp., *B. afzelii*, *Rickettsia* spp., and different *Bartonella* species. In line with these results, human infections with several of these pathogens have been reported in Austria, *i.e.*, CPXV, as an important OPV, *Leptospira* spp., *Borrelia* spp., and *Rickettsia* of the SFG group (Stanek et al. 2009, Glatz et al. 2010, Radl et al. 2011, Sonnleitner et al. 2012). Due to the lack of data, the impact on human health of LCMV, TULV, DOBV, *Bartonella* spp., and *B. grahamii* detected in rodents in this part of Austria requires increased awareness of the Austrian physicians.

PUUV was identified as causative agent in some patients from Austria, but no clinical cases have been reported for Lower Austria, although this virus was detected in bank voles in that area (Aberle et al. 1999, Plyusnina et al. 2006). The failure to detect PUUV in our sample of bank voles may indicate that this virus was absent at the investigated sites in 2008 or present at a very low prevalence, even though a relatively high number of human hantavirus cases was detected that year in Austria ($n = 33$; Heyman et al. 2011). The detection of TULV in common voles and their similarity to other Austrian TULV sequences confirmed the circulation of this hantavirus in Austria (Bowen et al. 1997). For further analysis on the phylogeography and molecular evolution of TULV, future investigations should target not only the S but also the M segment. Importantly, the potential pathogenicity of this hantavirus needs additional studies in human patients and risk groups (Mertens et al. 2011). To confirm the presence of DOBV in Austria, as indicated by our observation of DOBV-reactive antibodies in a yellow-necked field mouse, reservoir studies and a molecular identification of the DOBV genotype are required.

We have confirmed herein that wood mice from Austria are susceptible to LCMV or closely related arenaviruses, as has already been shown for wood mice from Spain (Ledesma et al. 2009). In contrast to previous investigations in Europe (Kallio-Kokko et al. 2006), we did not find hints for LCMV infection in yellow-necked field mice, bank voles, and common voles. The observed low OPV prevalence in rodents contrasts the high prevalences of OPV-reactive antibodies in different rodent species reported in previous studies for other parts of Central Europe (Essbauer et al. 2009, Kinnunen et al. 2011).

The proportion of *Leptospira*-positive rodents and the presence of several *Leptospira* species in different rodent species is in accordance with previous studies (Sebek et al.1989). The detection of four different *Bartonella* spp. in our study confirmed the presence of these bacteria in Central Europe (Telfer et al. 2007, Kaiser et al. 2011, Janecek et al. 2012). *B. taylorii* was the most frequently detected species without apparent host specificity. In contrast, we found *B. grahamii* only in wood mice, although it has been shown in many small sylvatic mammals (Holmberg et al. 2003). *B. doshiae* was detected solely in bank voles, supporting previous observations in Slovenia (Knap et al. 2007). In accordance with its first description in *Apodemus* spp. (Bermond et al. 2000), *B. birtlesii* was only found in wood mice.

Nearly 15% of our sampled rodents were infected by *B. afzelii.* Although specific rodent-associated genospecies may be better adapted to particular rodent species (Richter et al. 2004a,b, Richter et al. 2011), we observed no specificity in our samples. Presence of *Borrelia* DNA in the skin fails to prove reservoir status, but demonstrates contact with an infected tick. This might be the case for the common vole in which DNA of bird-associated *B. garinii* was detected. Information on the role of rodents in the natural cycle of different *Rickettsia* species is limited. Epidemiological data mostly based on questing ticks revealed the presence of several species of the SFG group in Austria (Blaschitz et al. 2008, Dobler et al. 2008). Detection of rickettsia DNA and rickettsia-specific antibodies in our study confirmed results previously reported for Bavarian rodents (Schex et al. 2011).

In contrast to reports of Q fever infection in humans (Kaplan and Bertagna 1955, Allenberger et al. 2009) and rodents in Tyrol (Stützner et al. 1979), we failed to detect C. *burnetii*. The occurrence of *C. burnetii* in rodents seems to be related to anthropogenic impact, such as farming of goats, cattle, and sheep (Webster et al. 1996, Reusken et al. 2011). In contrast, the agent was not detected in rodents inhabiting sylvatic sites (Reháček et al. 1993). The failure to detect *T. gondii* in rodent samples was not unexpected because a large study conducted in the Czech Republic examining rodents as potential intermediate hosts revealed a prevalence of only 0.9% viable *T. gondii* in 5166 small mammals of 17 species (Hejlíček and Literak 1998). Older rodents and rodents trapped close to dwellings are more likely to have seroconverted (Dabritz et al. 2008). Thus, in our study, the character of the trapping site and age of the trapped rodents may have influenced the likelihood to detect *C. burnetii* and *T. gondii* infection.

Information on multiple infections in rodents is sparse. In our study, we found seven of 110 (6.4%) of the animals infected by two pathogens and additionally two of 110 (1.8%) by three. In a study on 44 rodents in Croatia, dual infections with hantaviruses and *Leptospira* (16%), hantaviruses and *Babesia* (5%), and *Leptospira* and *Babesia* (2%), and triple infections in 7% of the rodents were demonstrated (Tadin et al. 2012). Moreover, interactions of pathogens, *i.e.*, of CPXV, *Babesia microti*, *Bartonella* spp., and *Anaplasma phagocytophilum*, have been identified in field voles (Telfer et al. 2010).

Conclusions

In summary, we demonstrate in our pilot study at two selected sample sites that multiple rodent-associated pathogens occur in Austria. Despite the relatively low number of

collected and tested animals, we detected several pathogens with zoonotic potential. Also, coinfections with more than one pathogen do not seem uncommon in wildlife. Thus, our results indicate that rodents may be able to transmit a multitude of pathogens directly or indirectly to other animals or humans. Future investigations will have to examine the potential interactions of different pathogens, their influence on the reservoir competence and fitness of the host, and the underlying molecular mechanisms, as well as the potential public health impact of these multiple infections. Further studies also have to examine whether and which site-specific, seasonal, and annual variations of the prevalence within reservoir and transmission risk occur.

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No competing financial interests exist for any of the authors.

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