Parasites & Vectors

Rats as potential reservoirs for neglected zoonotic *Bartonella* species in Flanders, Belgium

Maria Krügel[1], Martin Pfeffer[1], Nina Król[1], Christian Imholt[2], Kristof Baert[3], Rainer G. Ulrich[4,5] and Anna Obiegala[1*]

Abstract

Background: *Bartonella* spp. are vector-borne pathogens transmitted to humans *via* blood-sucking arthropods. Rodents such as the black rat (*Rattus rattus*) and Norway rat (*R. norvegicus*) are thought to be the main reservoirs. An infection with rodent-associated *Bartonella* spp. may cause severe symptoms in humans such as endocarditis and neuroretinitis. The current knowledge of *Bartonella* prevalence in rats from western Europe is scarce.

Methods: Rats and a few other rodent by-catches were trapped in the context of a rodenticide resistance study at different sites in Flanders, Belgium. During dissection, biometric data were collected, and spleen tissues were taken. DNA was extracted from spleen samples and tested for *Bartonella* spp. by conventional generic polymerase chain reaction (PCR). To determine the *Bartonella* species, a selected number of amplicons were sequenced and compared with GenBank entries.

Results: In total, 1123 rodents were trapped. The predominate species was *R. norvegicus* (99.64%). Other rodents trapped included: two water voles (*Arvicola amphibius*, 0.18%); one colour rat (*R. norvegicus* forma *domestica*, 0.09%); and one muskrat (*Ondatra zibethicus*, 0.09%). PCR analysis of 1097 rodents resulted in 410 (37.37%, 95% CI: 34.50–40.31%) *Bartonella* spp. DNA-positive samples. *Bartonella tribocorum* (94.68%, 95% CI: 88.02–98.25%) was the most frequently detected *Bartonella* species, followed by *B. grahamii* (3.19%, 95% CI: 0.66–9.04%) and *B. doshiae* (1.06%, 95% CI: 0.03–5.79%). An uncultured *Bartonella* species occurred in one water vole (1.06%, 95% CI: 0.03–5.79%). There was a significantly higher *Bartonella* prevalence in older rats compared to juveniles and a significant difference in *Bartonella* prevalence concerning the localisation of trapping sites. In contrast, there was no statistically significant difference in *Bartonella* prevalence regarding sex, degree of urbanisation and season.

Conclusions: Based on the high prevalence found, we conclude that the Norway rat seems to be a key reservoir host for zoonotic *B. tribocorum* in Belgium.

Keywords: *Bartonella*, *Bartonella tribocorum*, *Bartonella doshiae*, *Bartonella grahamii*, Belgium, Rodents, *Rattus norvegicus*, Rats, Europe

Background

The genus *Bartonella* consists of haemotropic, facultative intracellular, gram-negative α-proteobacteria, which parasitize endothelial cells and erythrocytes of mammalian hosts [1, 2]. *Bartonella* spp. may cause different diseases such as cat scratch disease, trench fever and Oroya fever with various symptoms, e.g. endocarditis, regional swelling of lymph nodes and vasoproliferative lesions of the skin and abdominal organs [3, 4]. Thus, an undetected infection with these neglected pathogens and inadequate therapy may be life-threatening [5]. Arthropods often play an important role in the transmission of these pathogens; commonly the

*Correspondence: Anna.Obiegala@vetmed.uni-leipzig.de
[1] Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany
Full list of author information is available at the end of the article

Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 2 of 12

infectious agents are transmitted to humans *via* fleas (e.g. the cat flea, *Ctenocephalides felis* for *Bartonella henselae* and the rodent flea, *Ctenophthalmus nobilis* for *B. grahamii*), the body louse (*Pediculus humanus corporis*), sand fly (*Lutzomyia verrucarum*) or ticks (e.g. *Ixodes ricinus*) [6–10].

Although many different *Bartonella* species are known, only a few of them are pathogenic to humans [1, 5, 8, 11–15]. Rodents are thought to be the main reservoir for most *Bartonella* species; however, a majority of these are not zoonotic. The Norway rat (*Rattus norvegicus*) is known to harbour both non-pathogenic (e.g. *B. rattimasiliensis* and *B. taylorii*) as well as pathogenic *Bartonella* spp. [8, 12, 13]. While rat-associated *B. elizabethae*, *B. vinsonii arupensis* and *B. washoensis* may cause cardiac diseases, *B. grahamii* is suspected to induce neuroretinitis, and *B. tribocorum* may cause unspecific symptoms such as fever, apathy and chronic fatigue [8, 12, 13]. Being a reservoir for *Bartonella* spp. and other zoonotic agents, rodents are crucial for the transmission and maintenance of vector-borne pathogens [7, 9, 16]. In particular, Norway and black rats (*R. rattus*) may be of concern. As highly synanthropic rodents they inhabit buildings and households, live in close contact to humans in urban and suburban regions and feed on human spoilage [17]. Therefore, monitoring of vector-borne pathogens in connection with rat populations, serving as hosts and reservoirs, is an essential component for the surveillance, prevention and risk control in the context of public health management and One Health politics, especially concerning neglected pathogens such as *Bartonella* spp. [9, 18].

Many studies worldwide describe moderate to high *Bartonella*-infection rates in rodents [19, 20]. Seemingly, western Europe is an endemic region with high prevalence levels for *Bartonella* in rodents and their ectoparasites (France: 11–70% [21–23]; Denmark: 30–53% [24]). These studies mostly refer to wild mice and voles. So far, there are only two studies describing *Bartonella* prevalence rates in rats from western Europe (Marseille, France: 30.3% [21]; Paris, France: 53.5% [25]), whilst another study evaluated the presence of *Bartonella* spp. in Norway and black rats from different European countries [26]. The latter study also included a pilot investigation of 60 Norway rats from Belgium indicating a high prevalence of *B. tribocorum*. To further explore the topic of this pilot investigation, the present study aims to: (i) determine the prevalence of *Bartonella* spp. in Norway rats from Flanders, Belgium; (ii) identify *Bartonella* species in these rats; and (iii) analyse *Bartonella* prevalence rates regarding the sex and age of the rats, seasonal influence, geographical location and degree of urbanisation.
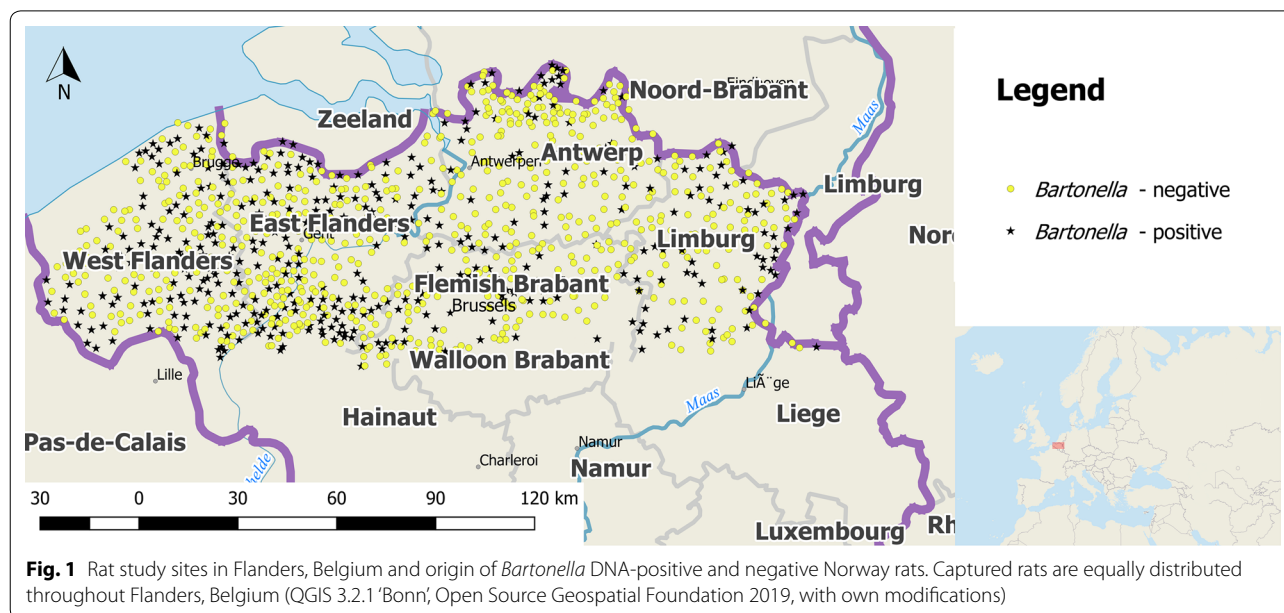
Methods

Study sites

The trapping sites are located in Flanders, the northern part of Belgium (Fig. 1). This region covers an area of 13,682 km^2 and is characterized by a mean human population density of 485 individuals/km^2 [27–29]. Belgium shows classical features of a western industrialised region with well-developed infrastructure as well as agriculture and extensive industry [30]; 97.9% of the population live in urban areas [31]. Only 13.4% of Flanders and Belgium's capital Brussels are planted with forest, mostly Scots pines (*Pinus sylvestris*) [27, 32].

Sampling of rodents

In 2015 and 2016 rodents were trapped in the context of a rodenticide resistance study for Norway rats conducted by the Belgian Research Institute for Nature and Forest (INBO, Brussels, Belgium). Detailed information on rodent trapping procedures was published elsewhere [33]. Rodent carcasses were kept frozen (− 20 °C) until necropsies were performed. Biometric data (body length, tail length, body weight and sex) were collected and spleen tissue was taken for further examination. A morphological identification key was used for species identification [34]; for a few individuals, molecular species identification and sex determination was performed. The age classification of rats was based on the body weight: all individuals with a body weight < 200 g were defined as juveniles and all individuals with a body weight ≥ 200 g were classified as adults [35].

DNA extraction

Spleen samples with a size of 1 × 0.5 × 0.2–0.4 cm were homogenized together with 0.6 g sterile ceramic beads (1.4 mm in size; PeqLab Biotechnologie GmbH, Erlangen, Germany) and 500 μl phosphate-buffered saline (PBS, pH 7.2). Homogenization of the tissues was carried out using a Precellys 24 Tissue Homogenizer (PeqLab Biotechnologie GmbH) at 5500 × *rpm*, twice for 15 s and a break of 10 s in between both runs. Lysis buffer and proteinase K (140 μl and 20 μl, respectively; QIAamp DNA mini kit (Qiagen, Hilden, Germany)) were added to each sample, followed by an overnight incubation at 56 °C in a thermomixer (Eppendorf, Hamburg, Germany). Subsequently, DNA extraction was performed manually with the QIAamp DNA Mini Kit (Qiagen) as recommended by the manufacturer. The quality and quantity of the extracted DNA samples were analysed spectrophotometrically using a NanoDrop ND-1000 (PeqLab Biotechnologie GmbH). Samples with a concentration of > 80 ng/μl DNA were diluted with water (bioscience grade, nuclease-free) to obtain a DNA concentration of 40–80 ng/μl for each sample.

Fig. 1 Rat study sites in Flanders, Belgium and origin of *Bartonella* DNA-positive and negative Norway rats. Captured rats are equally distributed throughout Flanders, Belgium (QGIS 3.2.1 'Bonn', Open Source Geospatial Foundation 2019, with own modifications)

Bartonella spp. DNA detection and rat species identification by conventional PCR and sequence analyses

DNA samples were tested for the presence of *Bartonella* spp. *via* conventional polymerase chain reaction (PCR) targeting the *gltA* gene [36]. Positive samples were further processed by an additional PCR targeting 453–780 bp of the 16S–23S rRNA intergenic spacer (ITS) region [2, 37]. Subsequent electrophoresis was performed for both gene targets on 2% agarose gels stained with HDGreen® Safe DNA Dye (Intas Science Imaging, Göttingen, Germany) and analysed under UV light using a Gel Doc 2000 transilluminator (Bio-Rad Laboratories GmbH, Life Science Group, München, Germany).

A selected number of amplicons of the expected size were further processed for *Bartonella* species identification. Based on an overview of species determination of randomly chosen *Bartonella*-positive samples, a selection algorithm was defined. Nineteen pairs of two females (the lightest and the heaviest) and two males (the lightest and the heaviest) were chosen in order to create an overview of sex as well as weight and accordingly age status. Additionally, *Bartonella* sequence analysis was performed for all positive muskrats (*Ondatra zibethicus*) and water voles (*Arvicola amphibius*).

Selected amplicons were purified manually with the NucleoSpin® Gel and PCR clean-up kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's instructions, analysed for quality and quantity as mentioned above and commercially sequenced with forward (Ba325s: 5′-CTT CAG ATG ATG ATC CCA AGC CTT CTG GCG-3′) and reverse primers (Ba1100as: 5′-GAA CCG ACG ACC CCC TGC

TTG CAA AGC-3′) (Interdisziplinäres Zentrum für Klinische Forschung, Leipzig, Germany). The obtained sequences were aligned and analyzed with BioNumerics (version 7.6; Applied Maths N.V., Sint Martens-Latem, Belgium) and compared with sequences in GenBank using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A selection of representative sequences ($n = 94$) were deposited in the GenBank database under the following accession numbers: MN244575-MN244667.

Molecular species identification was completed for rat samples which could not be identified morphologically. The conventional *cytochrome b* gene PCR protocols followed the protocols by Parson et al. [38] and Schlegel et al. [39]. Molecular sex determination based on a PCR approach previously described was carried out for a few individuals which were in insufficient condition for morphological sex determination [40, 41]. Visualisation of PCR products and examination of amplicons were performed as indicated above.

GIS analysis: the degree of urbanisation

Geocoordinates (World Geodetic System 1984) were taken during the capture of almost all rats. Hence, geographical information system (GIS) analyses tools were used to analyse the origin of rats (QGIS 3.2.1 'Bonn', Open Source Geospatial Foundation 2019). Census data of human population density (resolution 1 km²) [42] were added and both layers and related information were joined by location. The degree of urbanisation was defined by a population threshold, applied to the population grid cells, as belonging to one of three classes: "urban" (with > 1500 inhabitants/km²); "town"

Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 4 of 12

(with 300–1500 inhabitants/km^2); and "rural" (with < 300 inhabitants/km^2) [43, 44].

Statistical analysis

Confidence intervals (95% CI) with a standard error α = 0.05 for the prevalence of *Bartonella* spp., sex and species analysis were determined by the Clopper and Pearson method using GraphPad Software (Graph-Pad Software Inc., SanDiego, CA, USA). Chi-square test (sample size > 30) was conducted for testing the independence of *Bartonella* prevalence rates concerning sex, season, location and degree of urbanisation. *P*-values (probability) < 0.05 were considered to be significant and the degrees of freedom (*df*) were specified by default. Three aspects were analysed in more detail. Individual infection risk as a function of age was assessed using a generalized additive model (gam, binomial error distribution; package *gamm4*) with a weight smoother function. Additionally, a generalized linear model (glm, *lme4* package) with a binomial error distribution was used to evaluate if provinces differed within each class of urbanisation ("urban" was excluded due to the low sample size in each province). Estimated marginal means were calculated using the *emmeans* package and back-transformed for logit-scale to visualize infection probabilities. Analyses were performed using R software [45]. In addition, geographical foci in the distribution of *B. tribocorum* genotypes were investigated using a Chi-square test on proportions, where the number of positive samples per state for each genotype would be significantly different to equipartition.

Results

Animal collection

The vast majority of the 1123 trapped rodents were Norway rats (1119/1123; 99.64%, 95% CI: 99.09–99.90%). By-catches represented two water voles (0.18%, 95% CI: 0.02–0.64%), one color rat (*Rattus norvegicus* f. *domestica)* (0.09%, 95% CI: < 0.01–0.50%) and one muskrat (0.09%, 95% CI: < 0.01–0.50%).

PCR analyses for *Bartonella* spp. and analyses of *Bartonella* prevalence rates in connection with sex, age and degree of urbanisation

A total of 1097 out of 1123 (97.68%, 95% CI: 96.63–98.48%) rodents were tested using the *Bartonella* PCR. The remaining rats could not be investigated due to the absence of spleen tissue, high grade rotting and/or autolysis. From all 1097 examined rats, 410 (37.37%, 95% CI: 34.50–40.31%) were *Bartonella* DNA-positive (Table 1).

Bartonella DNA-positive samples were distributed all over Flanders (Fig. 1, Additional file 1: Table S1).

As illustrated in Table 1, analysis of the *Bartonella* prevalence between males and females revealed no statistically significant difference ($\chi^2 = 0.084$, $df = 1$, $P = 0.7722$).

There was also no significant difference in the prevalence of *Bartonella* spp. between individuals from rural areas, towns and urban areas ($\chi^2 = 3.167$, $df = 2$, $P = 0.2637$) and no significant difference was found between seasons regarding *Bartonella* prevalence rates ($\chi^2 = 3.668$, $df = 2$, $P = 0.2254$).

However, the prevalence of *Bartonella* in juvenile rats with a weight < 200 g was significantly lower than in adult rats ($\chi^2 = 11.365$, $df = 1$, $P = 0.0007$). This is mirrored in the results of *gam* (Fig. 2), where individual infection risk increases with weight, but remains static at around 260 g before slightly dropping again with increasing weight.

Interestingly, there were statistically significant differences in the prevalence of *Bartonella* between locations ($\chi^2 = 34.27$, $df = 4$, $P < 0.0001$). Analysis of differences between provinces for different degrees of urbanisation revealed that this difference was only detected within rural areas. Here, infection probabilities in Antwerp were significantly lower compared to Flemish Brabant, East and West Flanders (Fig. 3). This could not be detected in areas with a higher human population density (town).

Sequence analysis of *Bartonella*-positive samples

Sequencing of 94 *Bartonella*-positive samples (94/410; 22.93%, 95% CI: 18.94–27.31%) resulted in the detection of three *Bartonella* species (Table 2). *Bartonella tribocorum* (89/94; 94.68%, 95% CI: 88.02–98.25%) was the predominating species. Eighty-eight rodents positive for *B. tribocorum* were Norway rats and one additional positive animal was a colour rat (Table 2). *Bartonella grahamii* (3/94; 3.19%, 95% CI: 0.66–9.04%) was detected in one muskrat and two Norway rats, while *B. doshiae* and uncultured *Bartonella* sp. were each detected in one water vole (1/94; 1.06%, 95% CI: 0.03–5.79%) (Table 2).

As illustrated in Fig. 4, Norway rats infected with *B. tribocorum* were found to be broadly distributed. The two *B. grahamii* DNA-positive Norway rats originated from different parts of Flanders.

Three different *B. tribocorum* genotypes were defined according to the degree of similarity of 100% (group I), 99% (group II) or 96% (group III) to a sequence (GenBank: HG969192) used as a prototype (Table 3). *Bartonella tribocorum* genotype I (100% identity to GenBank: HG969192) was normally distributed ($\chi^2 = 2.75$, $df = 4$, $P = 0.6005$) in the five areas of Flanders (Limburg, Flemish Brabant, Antwerp, East Flanders and West

Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 5 of 12

Table 1 *Bartonella* prevalence correlating with sex, age, season and degree of urbanisation and location of rodents

Category of rodent collection	Total no. of collected individuals (*n*; % (95% CI))	No. of tested individuals	*Bartonella* spp.-positive samples (*n*; % (CI)]
Total	1123		410; 37.37% (34.50–40.31)
Sex (*N* = 1097)			
Male	647; 57.61% (54.66–60.53)	632	239; 37.82% (34.02–41.73)
Female	476; 42.26% (39.35–45.21)	465	171; 36.77% (32.38–41.34)
na	0	–	–
Age (*N* = 1094)			
Juvenile	271; 24.13% (21.66–26.74)	263	75; 28.52% (23.14–34.39)
Adult	849; 75.60% (72.98–78.09)	831	335; 40.31% (36.96–43.74)
na	3; 0.27% (0.06–0.97)	–	–
Season (*N* = 1025)			
Spring	400; 35.62% (32.81–38.50)	392	138; 35.20% (30.48–40.16)
Summer	12; 1.07% (0.55–1.86)	12	3; 25.00% (5.49–57.19)
Winter	639; 56.90% (53.95–59.82)	621	251; 40.42% (36.53–44.40)
na	72; 6.41% (5.05–8.01)	–	–
Urbanisation (*N* = 1089)			
Rural	749; 66.70% (63.85–69.45)	734	269; 36.65% (33.24–40.20)
Town	306; 27.25% (24.66–29.95)	300	122; 40.67% (35.06–46.46)
Urban	60; 5.34% (4.10–6.82)	55	16; 29.09% (18.70–42.21)
na	8; 0.71% (0.31–1.40)	–	–
Province (*N* = 1089)			
Limburg	160; 14.25% (12.25–16.43)	159	59; 37.11% (29.59–45.12)
Flemish Brabant	155; 13.8% (11.84–15.96)	153	53; 34.63% (27.14–42.75)
Antwerp	243; 21.64% (19.26–24.16)	233	53; 22.75% (17.53–28.67)
East Flanders	298; 26.54% (23.97–29.22)	294	126; 42.86% (37.13–48.73)
West Flanders	259; 23.06% (20.63–25.64)	250	116; 46.40% (40.09–52.79)
na	8; 0.71% (0.31–1.40)	–	–

Abbreviations: N, total number of tested individuals; na, not available due to missing body parts, high grade rotting and/or autolysis, or missing information in the database; CI, confidence interval

Flanders), whereas genotype II (χ^2 = 17.026, *df* = 4, *P* = 0.001911) and III (χ^2 = 10.444, *df* = 4, *P* = 0.03357) exhibited significant differences, cumulating in East and West Flanders (Fig. 5).

Discussion

This study analysed the prevalence of *Bartonella* spp. in Norway rats from Flanders, Belgium. All rats and a few by-catches were caught in the context of a rodenticide resistance study, which defines rats as a target species in western Europe [33]. The Norway rat is omnipresent in Europe, and since the 20th century has nearly completely displaced the black rat [34].

The overall *Bartonella* prevalence in the rodent population in this study was 37.4%. This comparatively high prevalence for *Bartonella* spp. corresponds well with recent data of a pilot study on 60 Norway rats from Belgium with a similarly high *Bartonella* prevalence (*ca.* 35% [26]). A lower but also moderate to high prevalence of *Bartonella*, was described in Norway rats from

Europe (France: 30.3% [21]) and other continents, such as North America (Canada: 25% [46]; USA: 25% [47]), South America (Brazil: 19% [48]) and Asia (Taiwan, China: 10.3%, [49]).

Bartonella tribocorum was the most frequently detected species in this study, with 94.7% of all positive samples sequenced. This result is in line with previous findings (89.6% [26]) and suggests that Norway rats are the main reservoir host for *B. tribocorum* [50], at least in Flanders, Belgium.

Bartonella tribocorum is known to be adapted to rats [8, 12] and to persist in infected erythrocytes without affecting the erythrocytes' natural life span of about 54–65 days [51]. After the erythrocytes' apoptosis, *B. tribocorum* is released into the bloodstream again in order to invade new erythrocytes for replication. Thus, *B. tribocorum* is able to infect about 1% of the erythrocytes in rats, persisting in the host for a long time without seriously harming the host [52, 53].
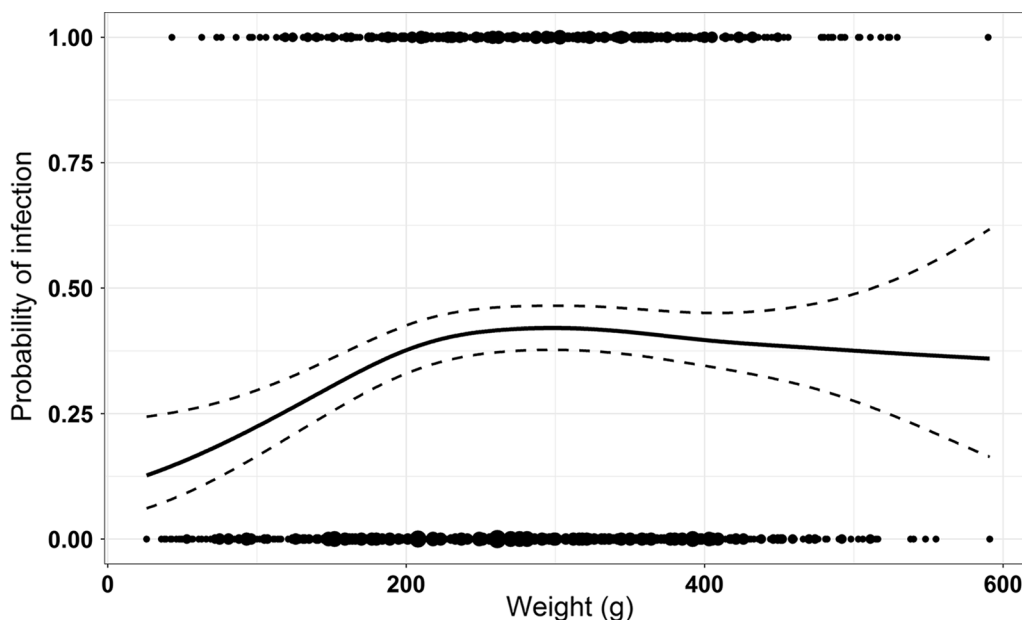
Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 6 of 12

Fig. 2 General additive model illustrating the probabilities of infection according to weight. The probability of detection of *Bartonella* DNA-positive individuals increases with increasing weight, cumulates at around 260 g and then slightly decreases with increasing weight
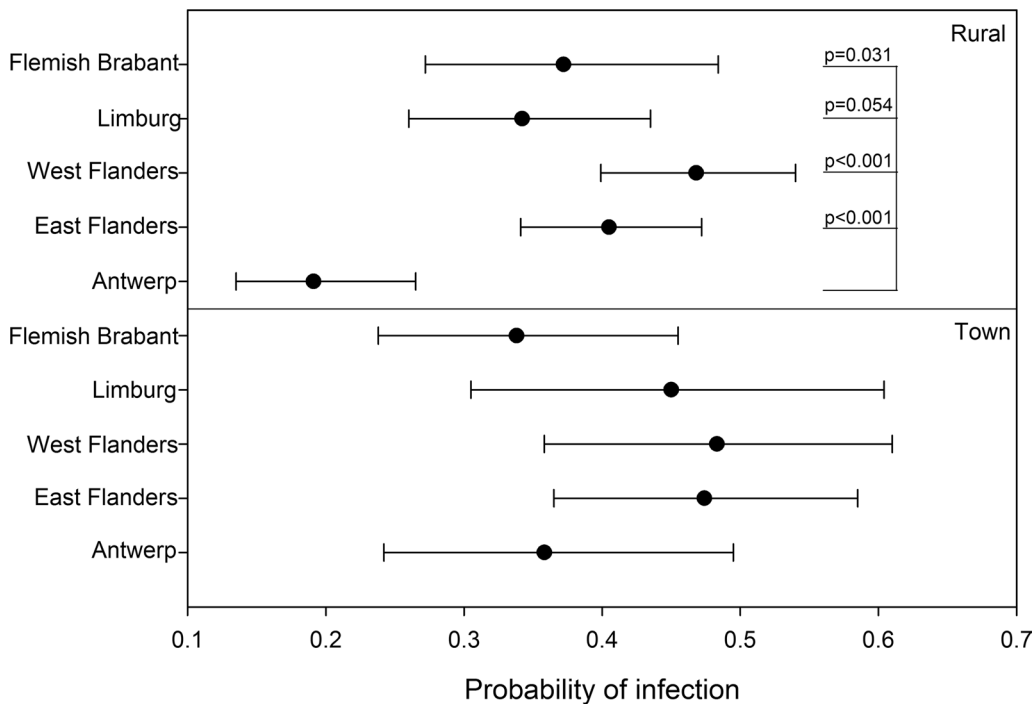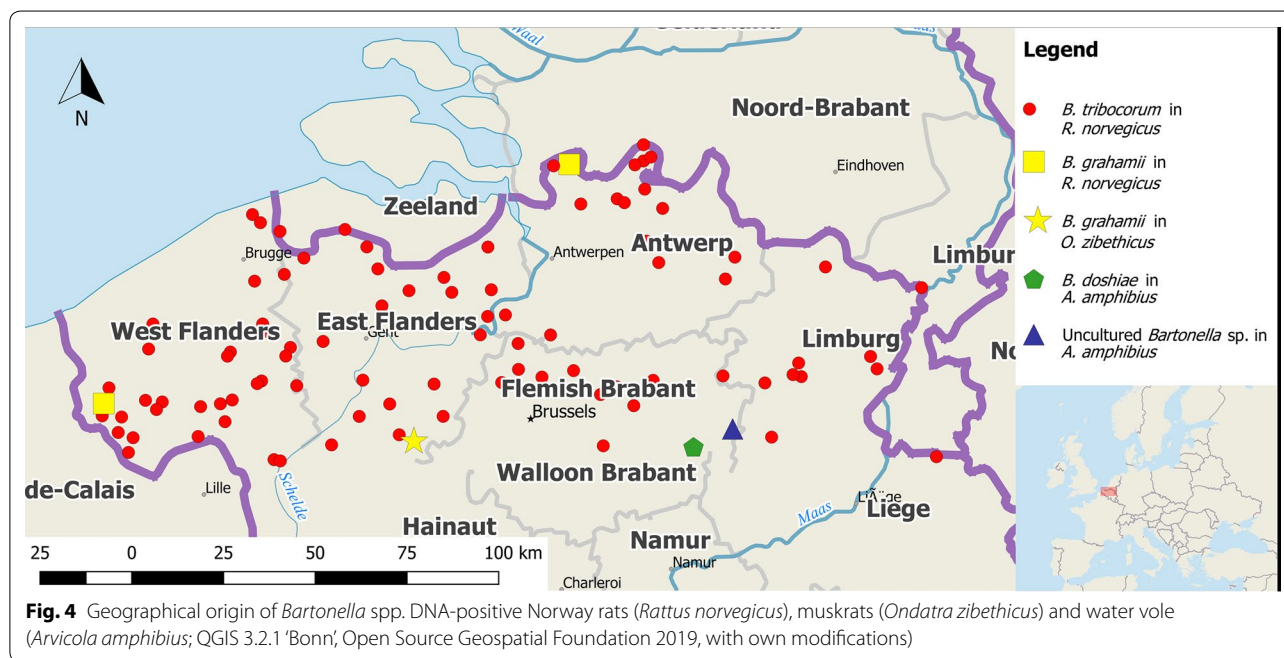

Fig. 3 Results of the binomial generalized linear model demonstrating probabilities of infection according to location and population density. Focussing on rural areas with a population density < 300 inhabitants/km^2, the probability of infection was significantly lower in Antwerp compared with East and West Flanders. This effect could not be demonstrated in areas with a higher human population density such as towns with 300–1500 inhabitants/km^2

Krügel *et al. Parasites Vectors* (2020) 13:235

Page 7 of 12

Table 2 *Bartonella* DNA detection in by-catch rodents relating to sex, age, season, urbanisation rate (habitat) and location

Species	n	Sex	Age class	Season	Habitat	Province	*Bartonella* spp.
Water vole (*Arvicola amphibius*)	2	Male (*n* = 1); female (*n* = 1)	Juvenile (*n* = 2)	na	Rural (*n* = 2)	Flemish Brabant (*n* = 2)	*B. doshiae* (*n* = 1); uncultured *Bartonella* sp. (Clone PD 125) (*n* = 1)
Muskrat (*O. zibethicus*)	1	Male	Adult	Summer	Rural	East Flanders	*B. grahamii*
Colour rat (*R. norvegicus* f. domestica)	1	Female	Adult	Winter	Town	Limburg	*B. tribocorum*

Abbreviations: n, total number; na, not available due to missing information in the database

Fig. 4 Geographical origin of *Bartonella* spp. DNA-positive Norway rats (*Rattus norvegicus*), muskrats (*Ondatra zibethicus*) and water vole (*Arvicola amphibius*; QGIS 3.2.1 'Bonn', Open Source Geospatial Foundation 2019, with own modifications)

There are only a few case reports of *B. tribocorum* infections in humans, and no reports of bartonellosis in Belgium. Displaying non-specific symptoms such as fever and apathy, *Bartonella*-infected humans are probably accidental hosts [9, 54]. Until now, there are just a few case reports describing bartonellosis as a zoonosis in central and western Europe. One study refers to six patients in France suffering from different unspecific symptoms; *B. tribocorum* was detected in two of these patients [55].
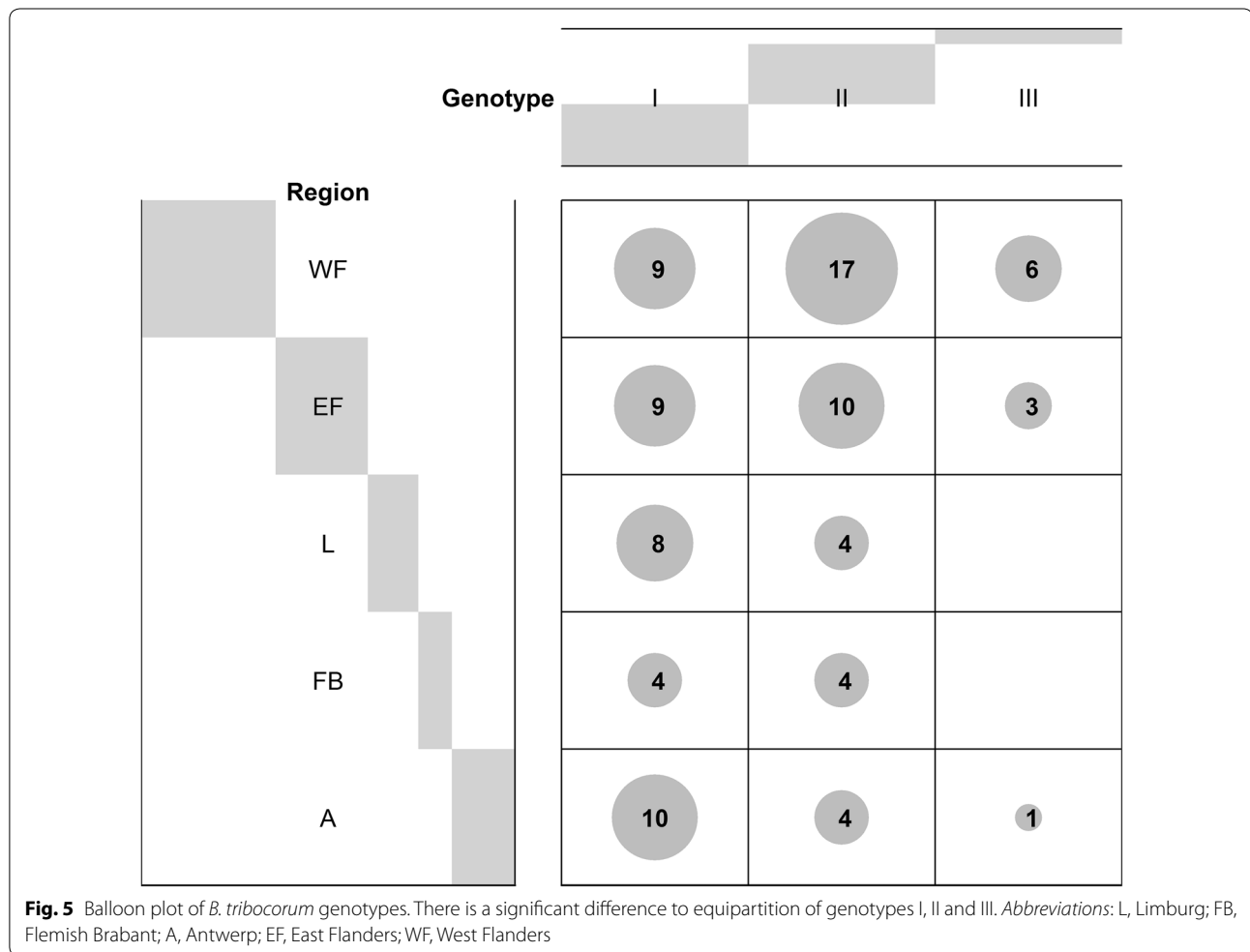
In the present study, *B. grahamii* and *B. doshiae* occurred in a very small number of samples. However, both species have been frequently reported in Europe [56]. *Bartonella grahamii* was detected in two *R. norvegicus* and one *O. zibethicus* in the present study. These results are in line with findings of *B. grahamii* in voles and mice from Europe [8, 10, 11]. The occurrence of *B. grahamii* in rats like *R. norvegicus* is uncommon but not completely unexpected as this pathogen was previously detected in Norway rats from Taiwan [57]. However,

to our knowledge, *B. grahamii* has not yet been identified in rats from Europe. The occurrence of *B. grahamii* in *O. zibethicus* was an unexpected finding, as blood parasites have been thus far rarely found in muskrats [58–60]. Further, muskrats are known to be rather insignificant hosts for *Bartonella* vectors such as ticks, fleas or lice (*Pediculus humanus corporis*) [61]. They usually act as hosts for hematophagous mites such as *Laelaps multispinosa*, *Zibethacarus ondatrae* and *Listrophorus* spp. [62, 63]. Possible reasons for the very low infestation with ectoparasites are the predominantly aquatic habitat of muskrats and their very dense fur [60]. To the best of our knowledge, this is the first detection of *Bartonella* species in a muskrat. As noted above, all *Bartonella* spp. are rather host-specific, with some *Bartonella* spp. being more host-specific than others. Host specificity of *Bartonella* spp. is closely related associated with the ability of adhering to and invading a host cell [64].

Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 8 of 12

Table 3 Sequence similarity of *Bartonella* spp. sequences detected in 89 Norway rats and one colour rat in Flanders, Belgium

Bartonella spp.	Identity to GenBank ID		No. of positive individuals	Proportion of individuals to different identities (%) (95% CI)
B. tribocorum	HG969192	100%	40	44.94 (34.38–55.86)
		99%	39	43.82 (33.32–54.75)
		96%	10	11.24 (5.52–19.69)
B. grahamii	CP001562	97%	2	100 (15.81–100)

Abbreviation: CI, confidence interval

Fig. 5 Balloon plot of *B. tribocorum* genotypes. There is a significant difference to equipartition of genotypes I, II and III. *Abbreviations*: L, Limburg; FB, Flemish Brabant; A, Antwerp; EF, East Flanders; WF, West Flanders

Bartonella grahamii possesses a higher number of genes for host-adaptability than human-associated bartonellae. These genes are located in a dynamic region being involved in a phage-derived run-off replication. This results in a high number of amplified genes associated with host-adaptation and a rapid diversification of these genes, enabling rodent-associated *Bartonella* species like *B. grahamii* to host shifts [64, 65]. Perhaps that is why *B. grahamii* was detected in a muskrat and

is adapted to various host species such as *Microtus* spp., *Myodes* spp. or *Apodemus* spp. [8]. Furthermore, *B. grahamii* is a zoonotic agent causing more specific and severe symptoms such as neuroretinitis [8, 13].

Until now, *B. doshiae* has not been associated with clinical human cases and thus is considered as non-pathogenic to humans [8]. *Bartonella doshiae* is a common European *Bartonella* species known to infect different *Rattus* species [66], voles of the genus *Microtus* [8, 56]

Krügel *et al. Parasites Vectors* (2020) 13:235

Page 9 of 12

and bank voles (*Myodes glareolus* [67, 68]). Hence, the occurrence of *B. doshiae* in one water vole was not unexpected, although prevalence studies of *Bartonella* spp. in water voles are lacking.

Three different sequence types I, II and III with 96%, 99%, 100% identity, respectively to the 16S-23S rRNA ITS region reference sequence of *B. tribocorum* (GenBank: HG969192) were detected. Individuals with sequence types II and III cumulated in the western provinces of Flanders (East Flanders and West Flanders; see Fig. 3). Whereas the knowledge about different sequence types in *B. tribocorum* is scarce, far more is known about sequence types (ST) in *B. henselae*. There are 118 different *B. henselae* strains, which were classified into 12 to 14 STs [69, 70]. Arvand et al. [70] confirmed the association between different STs of *B. henselae* and different hosts: most human pathogenic *B. henselae* isolates (66%) from Europe were identified as ST1, while most feline isolates (27.2%) belong to ST7. Sequence types, however, differed regionally between the UK and Spain (UK, ST2; Spain, ST1) in humans [69, 71].

The occurrence and host specificity of the sequence types of *B. henselae* seem to depend on the geographical location. Besides, coevolutionary processes have been observed between *Bartonella* spp. and rodent hosts [72]. Possibly the accumulation of the sequence types II and III in West Flanders results from geographical distribution patterns or from a coevolution with the mammalian host. A combination of the two seems also possible.

Moreover, we found that the sex of rodents had no influence on the prevalence of *Bartonella*. Kosoy et al. [73] came to the same conclusion, examining the relationship of *Bartonella* prevalence and sex in a wild cotton rat (*Sigmodon hispidus*) population from Georgia, USA. In contrast, the age of rats plays an important role for the individual infection status with *Bartonella* in Norway rats. In the present study, juvenile animals were significantly less frequently *Bartonella* DNA-positive (Table 1 and Fig. 4). In contrast to our result, investigations of wild cotton rats and American bush rats (*Neotoma micropus* and *N. albigula*) showed that juveniles had a significantly higher prevalence of *Bartonella* than adults [73, 74]. Additionally, a study of small mammals (*Myodes* spp., *Apodemus* spp., *Microtus* spp. and *Sorex* spp.) in central Europe concluded that there is no significant effect of age on individual infection probability in any of the small mammal species [20]. However, those studies investigated different species and the methodology differed from the present study.

A possible explanation as to why older animals are significantly more likely to be *Bartonella* DNA-positive could be found in the infectious life-cycle of *B.*

tribocorum. This species can persist in the host until its death without fatally damaging it [52, 53].

In our study, no significant differences in *Bartonella* prevalence rates according to season were found, although comparable studies demonstrate those for cotton rats from the USA where a very high infection level was reached in autumn (95%) with a lower prevalence in early summer (49%) [73]. This discrepancy might be explained by the distinct families of tested rodents, different study design and in particular by the absence of rat trapping in the autumn in our study.

Statistical analysis revealed the province of Antwerp exhibiting a significantly lower prevalence of *Bartonella* spp. in rural areas compared to East and West Flanders. The reason behind this lower prevalence remains speculative. However, the province of Antwerp, with a gross domestic product of € 80,981 million (2016, Data Explorer [75]), is one of Flanders most powerful economic and industrial regions with consequences on natural habitats and the absence of vectors, particularly in rural areas.

The retrospective data analysis of *Bartonella* prevalence according to degree of urbanisation was limited (unbalanced group sizes). Nevertheless, the results of this study are in line with Obiegala et al. [26], who demonstrated no significant differences of *Bartonella* prevalence according to the human population density.

Although other authors have described a high prevalence of *Bartonella* spp. (53.5%) in an urban Norway rat population from western Europe (France, Paris [25]), we detected a lower, but still quite high, prevalence of *Bartonella* spp. (37.4%).

The group size of urban rats was the smallest compared to the town and rural rats but is of particular importance for the transmission of zoonoses [17]. Hence, urban rats live in close vicinity to humans and act as reservoirs for many other human pathogenic agents such as *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis*, as well as *Leptospira interrogans*, *Rickettsia typhi*, *Streptobacillus moniliformis* and Seoul orthohantavirus [33, 76].

Conclusions

An infection with *Bartonella* spp. is rarely diagnosed in European countries and bartonellosis became one of Europe's most neglected diseases. However, the results of the present study and other studies [20–24] reinforce that *Bartonella* spp. pose a real threat to human health. The high overall prevalence of *Bartonella* spp. (37.4%) reported here proves that there are human pathogenic *Bartonella* species in rats from Belgium. Our results suggest that rats of the species *R. norvegicus* seem to be a major reservoir host for *Bartonella* spp., especially for *B. tribocorum*, in Flanders, Belgium.

Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 10 of 12

Supplementary information

> **Additional file 1: Table S1.** PCR results for *Bartonella* spp. and trapping number with coordinates of trapping sites (N, North; E, East) per collected rodent, Belgium, 2015–2016.

Abbreviations

BLASTn: Basic Local Alignment Search Tool for nucleotides; bp: base pairs; CI: confidence interval; df: degrees of freedom; DNA: deoxyribonucleic acid; gam: generalized additive model; GIS: geographical information system; ITS: intergenic spacer; PBS: phosphate-buffered saline; PCR: polymerase chain reaction; rpm: rounds per minute; rRNA: ribosomal ribonucleic acid; ST: Sequence type; UV: Ultraviolet.

Authors' contributions

AO, MP and MK elaborated the study concept and design. KB, MK and AO collected the data. MK, AO and NK performed the experiments. CI and MK performed data analysis and visualization. MK wrote a draft of the manuscript. AO, MP, NK, RGU, CI and KB reviewed the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The data supporting the findings of this study are included within this article and its additional file. Raw datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. Representative sequences were submitted to the GenBank database under the accession numbers MN244575-MN244667.

Ethics approval and consent to participate

Rat trapping was performed by trained pest controllers with special technical knowledge. Norway rats are regarded as a major pest species in Europe. In order to control the population in Flanders, Belgium, trapping and killing of the rats did not require a legal permission. Other captured species mentioned below were presumed to be by-catch. Detailed information has been published elsewhere [33].

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany. [2] Julius Kühn-Institute, Federal Research Institute for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Münster, Belgium. [3] Research Institute for Nature and Forest, Brussels, Belgium. [4] Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany. [5] German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Insel Riems, Germany.

References

1. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Vet Res. 2005;36:383–410.
2. Schorn S, Pfister K, Reulen H, Mahling M, Silaghi C. Occurrence of *Babesia* spp., *Rickettsia* spp. and *Bartonella* spp. in *Ixodes ricinus* in Bavarian public parks, Germany. Parasit Vectors. 2011;4:135.
3. Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N Engl J Med. 1990;323:1573–80.
4. Koehler JE, Tappero JW. Bacillary angiomatosis and bacillary peliosis in patients infected with human immunodeficiency virus. Clin Infect Dis. 1993;17:612–24.
5. Prutsky G, Domecq JP, Mori L, Bebko S, Matzumura M, Sabouni A, et al. Treatment outcomes of human bartonellosis: a systematic review and meta-analysis. Int J Infect Dis. 2013;17:e811–9.
6. Brinkerhoff RJ, Kabeya H, Inoue K, Bai Y, Maruyama S. Detection of multiple *Bartonella* species in digestive and reproductive tissues of fleas collected from sympatric mammals. ISME J. 2010;4:955–8.
7. Rizzoli A, Silaghi C, Obiegala A, Rudolf I, Hubálek Z, Földvári G, et al. *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Front Public Health. 2014;2:251.
8. Vayssier-Taussat M, Le Rhun D, Bonnet S, Cotté V. Insights in *Bartonella* host specificity. Ann N Y Acad Sci. 2009;1166:127–32.
9. Tomassone L, Berriatua E, de Sousa R, Duscher GG, Mihalca AD, Silaghi C, et al. Neglected vector-borne zoonoses in Europe: into the wild. Vet Parasitol. 2018;251:17–26.
10. Silaghi C, Pfeffer M, Kiefer D, Kiefer M, Obiegala A. *Bartonella*, rodents, fleas and ticks: a molecular field study on host-vector-pathogen associations in Saxony, eastern Germany. Microb Ecol. 2016;72:965–74.
11. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis. 2006;12:389–94.
12. Ellis BA, Regnery RL, Beati L, Bacellar F, Rood M, Glass GG, et al. Rats of the genus *Rattus* are reservoir hosts for pathogenic *Bartonella* species: an Old World origin for a New World disease? J Infect Dis. 1999;180:220–4.
13. Inoue K, Maruyama S, Kabeya H, Yamada N, Ohashi N, Sato Y, et al. Prevalence and genetic diversity of *Bartonella* species isolated from wild rodents in Japan. Appl Environ Microbiol. 2008;74:5086–92.
14. Maggi RG, Mozayeni BR, Pultorak EL, Hegarty BC, Bradley JM, Correa M, Breitschwerdt EB. *Bartonella* spp. bacteremia and rheumatic symptoms in patients from Lyme disease-endemic region. Emerg Infect Dis. 2012;18:783–91.
15. Maguiña C, Gotuzzo E. Bartonellosis. Infect Dis Clin N Am. 2000;14:1–22.
16. Woolhouse MEJ, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. Emerg Infect Dis. 2005;11:1842–7.
17. Feng AYT, Himsworth CG. The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). Urban Ecosyst. 2014;17:149–62.
18. Cunningham AA, Daszak P, Wood JLN. One health, emerging infectious diseases and wildlife: two decades of progress? Philos Trans R Soc Lond B Biol Sci. 2017;19:372.
19. Gutiérrez R, Krasnov B, Morick D, Gottlieb Y, Khokhlova IS, Harrus S. *Bartonella* infection in rodents and their flea ectoparasites: an overview. Vector Borne Zoonotic Dis. 2015;15:27–39.
20. Obiegala A, Jeske K, Augustin M, Król N, Fischer S, Mertens-Scholz K, et al. Highly prevalent bartonellae and other vector-borne pathogens in small mammal species from the Czech Republic and Germany. Parasit Vectors. 2019;12:332.
21. Gundi VAKB, Davoust B, Khamis A, Boni M, Raoult D, La Scola B. Isolation of *Bartonella rattimassiliensis* sp. nov. and *Bartonella phoceensis* sp. nov. from European *Rattus norvegicus*. J Clin Microbiol. 2004;42:3816–8.
22. Buffet JP, Pisanu B, Brisse S, Roussel S, Félix B, Halos L, et al. Deciphering *Bartonella* diversity, recombination, and host specificity in a rodent community. PLoS ONE. 2013;8:e68956.
23. Buffet JP, Marsot M, Vaumourin E, Gasqui P, Masséglia S, Marcheteau E, et al. Co-infection of *Borrelia afzelii* and *Bartonella* spp. in bank voles from a suburban forest. Comp Immunol Microbiol Infect Dis. 2012;35:583–9.

Krügel *et al. Parasites Vectors*   (2020) 13:235

Page 11 of 12

24. Engbaek K, Lawson PA. Identification of *Bartonella* species in rodents, shrews and cats in Denmark: detection of two *B. henselae* variants, one in cats and the other in the long-tailed field mouse. APMIS. 2004;112:336–41.

25. Desvars-Larrive A, Pascal M, Gasqui P, Cosson JF, Benoît E, Lattard V, et al. Population genetics, community of parasites, and resistance to rodenticides in an urban brown rat (*Rattus norvegicus*) population. PLoS ONE. 2017;12:e0184015.

26. Obiegala A, Heuser E, Ryll R, Imholt C, Fürst J, Prautsch LM, et al. Norway and black rats in Europe: potential reservoirs for zoonotic arthropod-borne pathogens. Pest Manag Sci. 2019;75:1556–63.

27. van Landuyt W, Vanhecke L, Hoste I, Bauwens D. Do the distribution patterns of vascular plant species correspond to biogeographical classifications based on environmental data? A case study from northern Belgium. Landsc Urban Plan. 2011;99:93–103.

28. Strubbe D, Matthysen E. Predicting the potential distribution of invasive ring-necked parakeets *Psittacula krameri* in northern Belgium using an ecological niche modelling approach. Biol Invasions. 2009;11:497–513.

29. Bevolking: omvang en groei. https://www.statistiekvlaanderen.be/bevolking-omvang-en-groei#in_de_vlaamse_ruit_wonen_meeste_inwoners_per_km%C2%B2. Accessed 19 Apr 2019.

30. Maes D, van Dyck H. Butterfly diversity loss in Flanders (north Belgium): Europe's worst case scenario? Biol Conserv. 2001;99:263–76.

31. Belgium - urbanization 2016. Statistic. https://www.statista.com/statistics/455783/urbanization-in-belgium/. Accessed 9 July 2018.

32. Verheyen K, Vanhellemont M, Stock T, Hermy M. Predicting patterns of invasion by black cherry (*Prunus serotina* Ehrh) in Flanders (Belgium) and its impact on the forest understorey community. Divers Distrib. 2007;13:487–97.

33. Rouffaer LO, Baert K, van den Abeele AM, Cox I, Vanantwerpen G, de Zutter L, et al. Low prevalence of human enteropathogenic *Yersinia* spp. in brown rats (*Rattus norvegicus*) in Flanders. PLoS ONE. 2017;12:e0175648.

34. Westheide W, Rieger G. Spezielle Zoologie. Teil 2: Wirbel- oder Schädeltiere: Spektrum Akademischer Verlag; 2009.

35. Webster JP, Ellis WA, Macdonald DW. Prevalence of *Leptospira* spp. in wild brown rats (*Rattus norvegicus*) on UK farms. Epidemiol Infect. 1995;1995:195–201.

36. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995;33:1797–803.

37. Maggi RG, Breitschwerdt EB. Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. J Clin Microbiol. 2005;43:1171–6.

38. Parson W, Pegoraro K, Niederstätter H, Föger M, Steinlechner M. Species identification by means of the *cytochrome b* gene. Int J Legal Med. 2000;114:23–8.

39. Schlegel M, Ali HS, Stieger N, Groschup MH, Wolf R, Ulrich RG. Molecular identification of small mammal species using novel *cytochrome b* gene-derived degenerated primers. Biochem Genet. 2012;50:440–7.

40. Aasen E, Medrano JF. Amplification of the Zfy and Zfx genes for sex identification in humans, cattle, sheep and goats. Biotechnology. 1990;8:1279.

41. Bryja J, Konecny A. Fast sex identification in wild mammals using PCR amplification of the Sry gene. Folia Zoologica. 2003;52:269–74.

42. European Commission EU. GEOSTAT—Eurostat. https://ec.europa.eu/eurostat/web/gisco/geodata/reference-data/population-distribution-demography/geostat#geostat11. Accessed 31 July 2019.

43. Heuser E, Fischer S, Ryll R, Mayer-Scholl A, Hoffmann D, Spahr C, et al. Survey for zoonotic pathogens in Norway rat populations from Europe. Pest Manag Sci. 2017;73:341–8.

44. Serrano Giné D, Russo A, Brandajs F, Pérez Albert MY. Characterizing European urban settlements from population data: a cartographic approach. Cartogr Geogr Inf Sci. 2016;43:442–53.

45. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for statistical computing; 2018.

46. Himsworth CG, Bai Y, Kosoy MY, Wood H, DiBernardo A, Lindsay R, et al. An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: is pathogen presence a reflection of global and local rat population structure? Vector Borne Zoonotic Dis. 2015;15:21–6.

47. Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, et al. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. MBio. 2014;5:e01933-14.

48. Costa F, Porter FH, Rodrigues G, Farias H, de Faria MT, Wunder EA, et al. Infections by *Leptospira interrogans*, Seoul virus, and *Bartonella* spp. among Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil. Vector Borne Zoonotic Dis. 2014;14:33–40.

49. Lin JW, Chen CY, Chen WC, Chomel BB, Chang CC. Isolation of *Bartonella* species from rodents in Taiwan including a strain closely related to '*Bartonella rochalimae*' from *Rattus norvegicus*. J Med Microbiol. 2008;57:1496–501.

50. Heller R, Riegel P, Hansmann Y, Delacour G, Bermond D, Dehio C, et al. *Bartonella tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. Int J Syst Bacteriol. 1998;48:1333–9.

51. Derelanko MJ. Determination of erythrocyte life span in F-344, Wistar, and Sprague-Dawley rats using a modification of the ([3H]DFP) method. Fundam Appl Toxicol. 1987;9:271–6.

52. Schülein R, Seubert A, Gille C, Lanz C, Hansmann Y, Piémont Y, Dehio C. Invasion and persistent intracellular colonization of erythrocytes. J Exp Med. 2001;193:1077–86.

53. Harms A, Dehio C. Intruders below the radar: molecular pathogenesis of *Bartonella* spp. Clin Microbiol Rev. 2012;25:42–78.

54. Breitschwerdt EB, Kordick DL. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13:428–38.

55. Vayssier-Taussat M, Moutailler S, Féménia F, Raymond P, Croce O, La Scola B, et al. Identification of novel zoonotic activity of *Bartonella* spp., France. Emerg Infect Dis. 2016;22:457–62.

56. Oliver MK, Telfer S, Piertney SB. Major histocompatibility complex (MHC) heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola terrestris*). Proc Biol Sci. 2009;276:1119–28.

57. Hsieh JW, Tung KC, Chen WC, Lin JW, Chien LJ, Hsu YM, et al. Epidemiology of *Bartonella* infection in rodents and shrews in Taiwan. Zoonoses Public Health. 2010;57:439–46.

58. Warwick T. The parasites of the muskrat (*Ondatra zibethica* L.) in the British Isles. Parasitology. 1936;28:395.

59. Grundmann AW, Tsai YH. Some parasites of the muskrat, *Ondatra zibethicus osoyoosensis* (Lord, 1863) Miller, 1912 from the Salt Lake Valley, Utah. Trans Am Microsc Soc. 1967;86:139.

60. Cross HB, Campbell-Palmer R, Girling S, Rosell F. The Eurasian beaver (*Castor fiber*) is apparently not a host to blood parasites in Norway. Vet Parasitol. 2012;190:246–8.

61. Chomel BB, Boulouis HJ, Breitschwerdt EB, Kasten RW, Vayssier-Taussat M, Birtles RJ, et al. Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. Vet Res. 2009;40:29.

62. Bauer CA, Whitaker JO. Ectoparasites of muskrats from Indiana with special emphasis on spatial distribution of coexisting mites of the genus *Listrophorus*. Am Midl Nat. 1981;105:112.

63. Rockett CL, Johnston SA. Ectoparasitic arthropods collected from some northern Ohio mammals. Great Lakes Entomol. 1988;21:147–9.

64. Vayssier-Taussat M, Le Rhun D, Deng HK, Biville F, Cescau S, Danchin A, et al. The Trw type IV secretion system of *Bartonella* mediates host-specific adhesion to erythrocytes. PLoS Pathog. 2010;6:e1000946.

65. Berglund EC, Frank AC, Calteau A, Vinnere Pettersson O, Granberg F, Eriksson AS, et al. Run-off replication of host-adaptability genes is associated with gene transfer agents in the genome of mouse-infecting *Bartonella grahamii*. PLoS Genet. 2009;5:e1000546.

66. Angelakis E, Raoult D. Pathogenicity and treatment of *Bartonella* infections. Int J Antimicrob Agents. 2014;44:16–25.

67. Birtles RJ, Hazel SM, Bennett M, Bown K, Raoult D, Begon M. Longitudinal monitoring of the dynamics of infections due to *Bartonella* species in UK woodland rodents. Epidemiol Infect. 2001;126:323–9.

68. Schmidt S, Essbauer SS, Mayer-Scholl A, Poppert S, Schmidt-Chanasit J, Klempa B, et al. Multiple infections of rodents with zoonotic pathogens in Austria. Vector Borne Zoonotic Dis. 2014;14:467–75.

69. Chaloner GL, Harrison TG, Coyne KP, Aanensen DM, Birtles RJ. Multilocus sequence typing of *Bartonella henselae* in the United Kingdom indicates that only a few, uncommon sequence types are associated with zoonotic disease. J Clin Microbiol. 2011;49:2132–7.

70. Arvand M, Feil EJ, Giladi M, Boulouis HJ, Viezens J. Multi-locus sequence typing of *Bartonella henselae* isolates from three continents reveals hypervirulent and feline-associated clones. PLoS ONE. 2007;2:e1346.

71. Gil H, Escudero R, Pons I, Rodríguez-Vargas M, García-Esteban C, Rodríguez-Moreno I, et al. Distribution of *Bartonella henselae* variants in patients, reservoir hosts and vectors in Spain. PLoS ONE. 2013;8:e68248.

72. Lei BR, Olival KJ. Contrasting patterns in mammal-bacteria coevolution: *Bartonella* and *Leptospira* in bats and rodents. PLoS Negl Trop Dis. 2014;8:e2738.

73. Kosoy M, Mandel E, Green D, Marston E, Childs J. Prospective studies of *Bartonella* of rodents. Part I. Demographic and temporal patterns in population dynamics. Vector Borne Zoonotic Dis. 2004;4:285–95.

74. Morway C, Kosoy M, Eisen R, Montenieri J, Sheff K, Reynolds PJ, Powers N. A longitudinal study of *Bartonella* infection in populations of woodrats and their fleas. J Vector Ecol. 2008;33:353–64.

75. Eurostat—Data Explorer. http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=nama_10r_3gdp&lang=de. Accessed 27 Oct 2018.

76. Himsworth CG, Jardine CM, Parsons KL, Feng AYT, Patrick DM. The characteristics of wild rat (*Rattus* spp.) populations from an inner-city neighborhood with a focus on factors critical to the understanding of rat-associated zoonoses. PLoS ONE. 2014;9:e91654.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.