Pathogen community composition and co-infection patterns in a wild community of rodents

Jessica L. Abbate[1,2,3], Maxime Galan[1], Maria Razzauti[1], Tarja Sironen[4], Liina Voutilainen[5], Heikki Henttonen[5], Patrick Gasqui[6], Jean-François Cosson[1,7], Nathalie Charbonnel[1,*]

[1] CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier, France.

[2] UMR MIVEGEC, IRD, CNRS, Univ Montpellier, Montpellier, France.

[3] UMR UMMISCO, IRD, UPMC, UMI, Bondy, France.

[4] Department of Virology, Faculty of Medicine, University of Helsinki, Helsinki, Finland.

[5] Natural Resources Institute Finland (LUKE), FI-00790 Helsinki, Finland.

[6] INRAE Clermont-Ferrand-Theix, Unité d'Épidémiologie Animale, Route de Theix, 63122 Saint Genès Champanelle, France.

[7] UMR BIPAR INRAE-ANSES-ENVA, Maisons-Alfort, France.

[*] Corresponding author:

E-mail address : nathalie.charbonnel@inrae.fr (N. Charbonnel)

Abstract

Rodents are major reservoirs of pathogens that can cause disease in humans and livestock. It is therefore important to know what pathogens naturally circulate in rodent populations, and to understand the factors that may influence their distribution in the wild. Here, we describe the incidence and distribution patterns of a range of endemic and zoonotic pathogens circulating among rodent communities in northern France. The community sample consisted of 713 rodents, including 11 host species from diverse habitats. Rodents were screened for virus exposure (hantaviruses, cowpox virus, Lymphocytic choriomeningitis virus, Tick-borne encephalitis virus) using antibody assays. Bacterial communities were characterized using 16S rRNA amplicon sequencing of splenic samples. Multiple correspondence (MCA), regression and association screening (SCN) analyses were used to determine the degree to which extrinsic factors contributed to pathogen community structure, and to identify patterns of associations between pathogens within hosts. We found a rich diversity of bacterial genera, with 36 known or suspected to be pathogenic. We revealed that host species is the most important determinant of pathogen community composition, and that hosts that share habitats can have very different pathogen communities. Pathogen diversity and co-infection rates also vary among host species. Aggregation of pathogens responsible for zoonotic diseases suggests that some rodent species may be more important for transmission risk than others. Moreover we detected positive associations between several pathogens, including *Bartonella*, *Mycoplasma* species, Cowpox virus (CPXV) and hantaviruses, and these patterns were generally specific to particular host species. Altogether, our results suggest that host and pathogen specificity is the most important driver of pathogen community structure, and that interspecific pathogen-pathogen associations also depend on host species.

Keywords (6max)

16S rRNA amplicon high throughput sequencing; Disease Ecology; Microbial Interactions; Pathobiome; Rodent reservoirs; Zoonoses

1. Introduction

Infectious diseases are among the most important global threats to biodiversity, wildlife and human health, and are associated with potential severe socioeconomic consequences (Daszak, Cunningham & Hyatt 2000; Smith, Sax & Lafferty 2006; Jones *et al.* 2008). Although combatting these risks is a main worldwide priority, our understanding of the processes underlying disease emergence still remains too limited for developing efficient prediction, prevention and management strategies. In humans, the majority of emerging pathogens originate as zoonoses from animal host populations in which they naturally circulate (Taylor, Latham & Woolhouse 2001; Jones *et al.* 2008). Thus, identifying the epidemiological features (e.g., prevalence, diversity, host specificity, geographic distribution) of zoonotic pathogen communities in their wild hosts, and the factors that influence pathogen occurrence in those communities, is as important to human health as it is to understanding the fundamentals of disease ecology (Garchitorena *et al.* 2017).

Both extrinsic and intrinsic factors can contribute to the composition of natural pathogen communities within and between wild animal species, populations and individuals. Extrinsic factors include geographic location, climate, periodicity of epidemic cycles and abiotic features influencing inter-specific transmission opportunities (e.g., (Harvell et al. 2002; Burthe et al. 2006; Poulin et al. 2012)). Intrinsic factors such as host species identity, sex, age, and body condition as well as genetic and immunogenetic features have also been intensively studied (e.g., (Beldomenico *et al.* 2008; Streicker *et al.* 2010; Streicker, Fenton & Pedersen 2013; Salvador *et al.* 2011; Charbonnel *et al.* 2014; Bordes *et al.* 2017)). Although less investigated, inter-specific ecological interactions (e.g., competition, facilitation) among pathogens within animal hosts are also likely to be an important intrinsic force in determining the composition of pathogen communities. Ecological interactions between free-living species are well-known to play a part in the distribution, abundance, and many other qualitative and quantitative features of populations; the application of this basic tenant of community ecology to pathogen incidence and expression of disease has become recognized as imperative for assessing both risks and potential benefits posed to human health,

agriculture, wildlife, and conservation (Pedersen & Fenton 2007). Simultaneous infection by multiple parasite species is ubiquitous in nature (Petney & Andrews 1998; Cox 2001; Moutailler *et al.* 2016). Interactions among co-infecting parasites may have important consequences for disease severity, transmission and community-level responses to perturbations (Jolles *et al.* 2008; Telfer *et al.* 2010). Henceforth, and through the advent of sequencing technologies in particular, it is now possible and essential to investigate disease emergence from a multi-host / multi-pathogen perspective (Galan *et al.* 2016), considering the potential influence of pathogen interactions on current and future disease distributions (Cattadori, Boag & Hudson 2008; Jolles *et al.* 2008; Budischak *et al.* 2015; Abbate *et al.* 2018).

Rodent communities are relevant models for developing this community ecology approach to disease distribution and emergence. They harbor a wide variety of pathogenic taxa (e.g., Bordes *et al.* 2013; Pilosof *et al.* 2015; Koskela *et al.* 2016; Diagne *et al.* 2017) and are important reservoir hosts of agents of zoonoses that have severe implications for human health. Han et al. (2015) have revealed that about 10% of the 2277 extant rodent species are reservoirs of 66 agents of zoonoses, including viruses, bacteria, fungi, helminths, and protozoa. They also described 79 hyper-reservoir rodent species that could be infected by multiple zoonotic agents. Among the most important zoonotic pathogens, we find *Echinococcus* spp., *Schistosoma* spp., *Toxoplasma* spp., *Trypanosoma* spp., *Yersinia pestis*, *Leptospirosa* spp., *Bartonella* spp., hantaviruses (e.g. Puumala virus) and arenaviruses (e.g. *Lassa virus*) in particular (https://www.gideononline.com/). Strong ecological interactions have been shown among some of these pathogenic taxa (Telfer *et al.* 2010; Kreisinger *et al.* 2015), as well as between micro- and macroparasites (eg., (Salvador *et al.* 2011; Ezenwa & Jolles 2015)). These interactions have been shown to be dependent on the landscape (or habitat features) from which hosts were sampled (Ribas Salvador *et al.* 2011; Guivier *et al.* 2014). In addition, rodents share a number of habitats with humans, including urban settings, agricultural lands, and forests, providing opportunities for human-rodent contact and pathogen transmission (Davis, Calvet & Leirs, 2005). Describing the distribution and composition of natural pathogen

communities in rodent populations, and determining the drivers behind pathogen associations, is imperative for understanding the risks they may pose for public health.

In this study, we analyzed the pathogen communities carried by rodent communities in a rural area of northern France, a region known to be endemic for several rodent-borne diseases including nephropathia epidemica (Puumala orthohantavirus (Sauvage *et al.* 2002)) and borreliosis (*Borrelia* sp., Razzauti *et al.* 2015). We investigated exposure histories (via the presence of antibodies) for several viruses (hantaviruses, cowpox virus, lymphocytic choriomeningitis virus, Tick-borne encephalitis virus) and current or recent exposure to bacterial pathogens (using high-throughput 16S metabarcoding). We described the pathogens detected, their prevalence in the community and their individual distributions among host populations. We then tested the role of extrinsic factors (e.g., habitat, host species, host age) in explaining variation in pathogen distributions, and for associations (non-random co-infection frequencies) between pathogens that might indicate intrinsic drivers (e.g., competition, facilitation) of pathogen community composition. We expected that host species and habitat would be the most important factors structuring pathogen community composition because most pathogens are largely host-specific, but those sharing habitats should also share opportunities for transmission (Davies & Pedersen 2008). After accounting for extrinsic factors, we expected to retrieve several pathogen-pathogen associations previously identified in the literature. This included i) positive associations between cowpox virus and *Bartonella* infections (*Microtus agrestis,* (Telfer *et al.* 2010)); ii) positive associations between distinct *Mycoplasma* species in mammalian hosts (Sykes *et al.* 2008; Tagawa *et al.* 2012; Fettweis *et al.* 2014; Volokhov *et al.* 2017)); iii) associations between *Bartonella* and hemotropic *Mycoplasma* species (both positive and negative associations, as well as experimental demonstration of dynamic interactions, have been described in *Gerbillus andersonii* (Eidelman *et al.* 2019)). Lastly, we also expected to find previously-undescribed associations due to the large bacteria and rodent dataset included in our study. All these associations were likely to differ between host species, as differences in host specificity are also

likely to be accompanied by differences in transmission dynamics and host responses to infection (Davies & Pedersen 2008; Singer 2010; Dallas, Laine & Ovaskainen 2019).

2. Materials and methods

2.1. Study area and host sampling

Rodent sampling was conducted over two years (Autumn 2010 & 2011) in rural habitats surrounding two villages (Boult-aux-Bois and Briquenay) in the Ardennes region of northern France (previously described in (Gotteland *et al.* 2014)). Sex and age (based on precise body measurements and classed as 'adult' for sexually mature animals and 'juvenile' for both juveniles and sexually-immature sub-adults) were recorded for each animal, a blood sample was taken for serological analyses, and animals were then euthanized using isofluorane inhalation. Spleens were taken and stored in RNA*later* Stabilizing Solution (Invitrogen) at -20°C. Species captured from the two sites included (family: Cricetidae) 195 *Arvicola scherman* (montane water vole), 10 *Microtus agrestis* (field vole), 66 *Microtus arvalis* (common vole), 203 *Myodes glareolus* (bank vole); and (family: Muridae) 43 *Apodemus flavicollis* (yellow-necked mouse), 156 *Apodemus sylvaticus* (wood mouse), 32 *Rattus norvegicus* (brown rat). These seven focal host species were collected from traps placed in distinct landscapes (henceforth referred to as host 'habitats') (Supporting Information Figure S1): *R. norvegicus* were found uniquely on farms, *Ar. scherman* and *Mi. arvalis* were found almost entirely in meadows, and the five remaining species occupied both forests and hedgerows. Demographic differences between host species were observed for sex (e.g., male bias in *Ap. sylvaticus*; Figure S2A) and age classes (e.g., relative abundance of juveniles in *Mi. arvalis* and *Ap. slyvaticus* hosts; Figure S2B). Five *Microtus subterraneus* (European pine vole) and one each of three additional host species (one cricetid and two murids) were also found in these communities, but excluded from analyses due to their rarity; these rare (non-focal) hosts and their pathogens are described in Supporting Materials Appendix S1.

150 *2.2. Detecting virus exposure and bacterial infection*

151 Among the 713 rodents sampled for this study, indirect fluorescent antibody tests (IFATs; see for
152 details (Kallio-Kokko *et al.* 2006)) were successfully performed on 677 animals to detect
153 immunoglobulin G (IgG) specific to or cross-reacting with cowpox virus (CPXV, *Orthopoxvirus*),
154 Puumala or Dobrava-Belgrade virus (respectively PUUV and DOBV, *Orthohantavirus*, collectively
155 referred to henceforth as "hantavirus"), lymphocytic choriomeningitis virus (LCMV,
156 *Mammarenavirus*), and Tick-borne encephalitis virus (TBEV, *Flavivirus*). We refer to these
157 antiviral antibody tests as indicating a history of past exposure, but hantavirus and LCMV
158 antibodies also likely indicate continued chronic infection. In contrast, current or very recent
159 exposure to bacterial infection was tested via 16S rRNA gene amplicon sequencing of splenic
160 tissue, giving no indication of past exposure history. Funding was available to test for bacteria in
161 just half of the animals, chosen haphazardly to equally represent all host species, study sites and
162 years, resulting in successful analysis for 332 rodents (see Figure 1 for a breakdown of number of
163 individuals sampled per focal host species). For each individual animal, the DNA from splenic
164 tissue was extracted using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer
165 recommendations. Each DNA extraction was analyzed twice independently. We followed the
166 method described in Galan *et al*. (2016) to perform PCR amplification, indexing, pooling,
167 multiplexing, de-multiplexing, taxonomic identification using the SILVA SSU Ref NR 119
168 database as a reference (http://www.arb-silva.de/projects/ssu-ref-nr/). Briefly, DNA samples were
169 amplified by PCR using universal primers targeting the hyper variable region V4 of the 16S rRNA
170 gene (251 bp) and sequencing via Illumina MiSeq. The V4 region has been proven to have
171 reasonable taxonomic resolution at the genus level (Claesson *et al.* 2010). A multiplexing strategy
172 enabled the identification of bacterial genera in each individual sample (Kozich *et al.* 2013). Data
173 filtering was performed as described in Galan et al. (2016) to determine presence/absence of
174 bacterial infections (summarized in Figure S3). Briefly, we discarded all bacterial OTUs containing
175 fewer than 50 reads in the entire dataset and animals for which one or both individual PCR samples

176 produced fewer than 500 reads. A bacterial OTU was considered present in an animal if the two
177 independent PCR samples were both above a threshold number of reads, defined as the greater of
178 either 0.012% of the total number of reads in the run for that OTU (i.e. filtering using the rate of
179 indexing leak) or the maximum number of reads for that OTU in any negative control sample (i.e.
180 filtering using the presence of reads in the negative controls due to contaminations) (Galan *et al.*
181 2016). For each OTU suspected as pathogenic, Basic Local Alignment Search Tool (BLAST)
182 searches of the most common sequences were conducted to infer species identity where possible.
183 OTUs with at least 500 reads across all animals in the dataset were considered reliably detectable,
184 allowing us to assign absent status to these OTUs in animals failing to meet the criteria for OTU
185 presence. Only OTUs for which there were at least 500 reads across all animals in the dataset (for
186 which present and absent statuses could be assigned), and where reasonable certainty of
187 pathogenicity could be established from the literature, were considered in analyses of the pathogen
188 community.
189
190 *2.3. Statistical Methods*
191 All statistical analyses were implemented in R version 3.2.2 (R Core Team 2015).
192
193 *2.3.1. Testing for extrinsic drivers of pathogen community composition across the rodent*
194 *community*
195 We analyzed pathogen community composition across the whole rodent community. We first
196 estimated pathogen community richness using the Shannon diversity index (alpha diversity)
197 considering bacterial OTUs and anti-viral antibodies found in each study year, study site, habitat,
198 host species, host sex and host age group. We evaluated a linear regression model using analysis of
199 deviance ('lm' and 'drop1' functions from the basic *stats* package) to test for significance of
200 differences in pathogen diversity due to the fixed factors listed above after first correcting for all
201 other factors in the model (marginal error tested against the *F*-distribution). Post-hoc comparisons

and correction for multiple tests were performed using function 'TukeyHSD' from package *stats* and 'HSD.test' from package *agricolae* to regroup significantly distinct factor levels.

We next tested for differences in pathogen community composition (beta diversity) between host species, habitats, study sites, years, age groups, and sexes. We used multiple correspondence analysis (MCA) to reduce variance in presence/absence of each bacterial pathogen species and anti-viral antibody, implemented with the function 'MCA' in the *FactoMinR* package and visualization tools found in the *factoextra* package. This produced a down to a set of quantitative and orthogonal descriptors (dimensions) describing the pathogen community composition. With each MCA dimension as a continuous dependent response variable, we then evaluated linear regression models using analysis of deviance with post-hoc comparisons as detailed above.

2.3.2. Detecting and testing for associations between co-circulating pathogens

Because we identified a large number of pathogens, the number of potential association combinations to consider was excessively high, especially with regard to the relatively small number of rodents sampled. We therefore decided to test the significance only of those associations (i) clearly suggested by the community-wide MCA or (ii) previously described in the literature: positive association between *Bartonella* spp. and CPXV (Telfer *et al.* 2010), positive associations between *Mycoplasma* species (Sykes *et al.* 2008; Tagawa *et al.* 2012; Fettweis *et al.* 2014; Volokhov *et al.* 2017), and both positive (Kedem *et al.* 2014; Eidelman *et al.* 2019) and negative (Cohen, Einav & Hawlena 2015) associations between *Bartonella* spp. and hemotropic *Mycoplasma* species. Given the *a priori* assumption that associations would differ between host species, we analyzed each host species separately; where evidence suggested no significant differences between host species, we pooled individuals into a single analysis to gain statistical power.

We tested the significance of each association using both association screening (SCN) analysis (Vaumourin *et al.* 2014) and multiple logistic regression analysis (GLMs, modeling the binomial

228 'presence/absence' status of each pathogen as a function of the occurrence of other pathogens). We
229 first performed SCN analysis, as this approach is among the most suitable for detecting pathogen
230 associations in cross-sectional studies (Vaumourin et al. 2014). Briefly, given the prevalence of
231 each pathogen species in the study population, SCN analysis generates a simulation-based 95%
232 confidence envelope around the expected frequency of each possible combination of concurrent
233 infection status (a total of 2^{NP} combinations, where NP = the number of pathogen species) under the
234 null hypothesis of random pathogen associations. Observed frequencies of co-infection
235 combinations falling above or below this envelope are considered to occur more or less frequently,
236 respectively, than in 95% of the random simulations. Significance of the association is given as a *p*-
237 value, calculated as the number of instances in which the simulated co-infection frequency differed
238 (above or below the upper or lower threshold, respectively) from the observed frequency divided by
239 the total number of simulations (Vaumourin *et al.* 2014).

240 The benefit of the SCN approach is a relatively high level of statistical power and the ability to
241 identify precisely which combinations of pathogens occur outside the random expectations
242 (Vaumourin et al. (2014)). However, the SCN is sensitive to heterogeneity in the data due to
243 extrinsic factors (e.g., host specificity, or structuring in space, time, age or sex), which can both
244 create and mask true associations. A multiple logistic regression (GLM) approach was thus also
245 systematically employed, as it has the benefit of explicitly taking into account potentially
246 confounding extrinsic factors. Binomial exposure (presence/absence of either bacterial infection or
247 anti-viral antibodies) to a single pathogen was set as the dependent variable with exposure to the
248 hypothetically associated pathogen(s) treated as independent variable(s) and extrinsic factors (host
249 sex, host age, study year, study site, and where appropriate, habitat) were specified as covariates
250 using function 'glm' in the *stats* package with a binomial logit link. When there was no *a priori*
251 assumption concerning timing of exposure (e.g. anti-viral anti-body presence is more likely to affect
252 current acute bacterial infection than the reverse), the occurrence of each pathogen implicated in a
253 given association was set as the dependent variable in reciprocal GLMs. As for the MCA

dimensions above, statistical significance of the association was assessed after first correcting for all covariates in the model using the 'drop1' function (-2 log likelihood ratio tests via single-term deletions compared to the full model). Despite a large number of *a priori* hypotheses, we regarded a p-value of < 0.05 as significant due to the very low number of individuals of each host species sampled. Though conceivably important, we also did not have sufficient power to test for interaction terms.

3. Results

3.1. Taxonomic identification and prevalence of pathogens

3.1.1. Viral exposure

The most abundant virus detected was CPXV, infecting 222 (32.8%) of the 677 animals tested. It was detected in all focal host species. However, significant variation in prevalence was observed between focal host species (highly prevalent (43-70%) in *Ar. scherman*, *Mi. agrestis*, and *My. glareolus*; Figure 1; χ^2=111.1, *df*=6, $p<10^{-4}$). Anti-hantavirus antibodies were detected in 16 animals (2.4%), and were significantly structured among host species (with exposure highest in *Mi. arvalis* (9.7%), *R. norvegicus* (3.3%) and *My. glareolus* (3.1%); Figure 1; χ^2=12.7, *df*=6, *p*=0.048). Anti-LCMV antibodies were detected in two *Mi. arvalis* individuals (Figure 1). No animals tested positive for anti-TBE antibodies.

3.1.2. Bacterial pathogens

Out of 952 bacterial OTUs represented by at least 50 reads in the dataset, 498 were considered positive in at least one animal after data filtering (presented in Supporting Information Table S1). We checked manually for potential chimera that would not have been removed from the dataset by the *Uchime* program implemented in *mothur.* Two OTUs (00024 & 00037) identified as *Bartonella* with low bootstrap values (74 and 92 respectively) appeared to represent chimeric sequences between the two highly amplified genera (*Bartonella* and *Mycoplasma*) in co-infected samples.

Two OTUs (00009 & 00117) which were unclassified but which had a large number of reads in positive animals were also found to represent chimeric sequences between the two genera, despite high bootstrap values (100). Three additional chimeric *Mycoplasma* OTUs with under 500 reads were also excluded (OTUs 00076, 00159, and 00316). Two OTUs (00002 & 00059) were found to be redundant with OTUs Myco1 and Myco3, respectively, and two more (00134 & 00220) were chimera between Myco OTUs. These 11 OTUs were manually removed from the database, and are not included in Table S1.

We identified 43 OTUs belonging to bacterial genera with members known or thought to be pathogenic in mammals (Table 1). After BLAST queries, we found 16 of these OTUs (representing 7 distinct genera) which could be considered as reliably detectable pathogens in the focal host species (Figure 1). An additional 24 OTUs were considered potentially pathogenic but excluded from analyses because they were only observed in rare host species, presence-absence could not be reliably established due to a low total number of reads (<500 in the dataset, e.g., *Borrelia* spp. and *Leptospira* spp.), inability to rule out contamination by natural sources of non-pathogenic flora during dissection (e.g., *Helicobacter* spp., *Streptococcus* spp.) or by known contaminants of sequencing reagents (e.g., *Williamsia* spp.; (Salter *et al.* 2014)), or because their identity to a pathogenic species was uncertain due to insufficient genetic variation at the 16S rRNA locus (e.g., *Yersinia* spp.) (Table 1). We also identified three OTUs belonging to the eukaryotic family Sarcocystidae (98% sequence similarity to the coccidian parasite *Sarcocystis muris*); though each OTU was represented by >500 reads, there are currently no data on the reliability of this method for detection (Table 1). Individual infection status for each of these OTUs is given in Table S2.

The 16 reliably detectable pathogenic OTUs included *Bartonella* spp., 10 *Mycoplasma* spp. OTUs, *Rickettsia canadensis*, "*Candidatus* Neoehrlichia mikurensis", *Orientia* spp., *Brevinema andersonii*, and *Spiroplasma* spp. Phylogenetic analysis including published sequences from BLAST queries revealed that the 10 *Mycoplasma* spp. OTUs belonged to three distinct species: *M. haemomuris* (Myco1-3,5,7-9), *M. coccoides* (Myco4 and Mco6), and "*Candidatus* M.

ravipulmonis" (Myco10) (Figure S4). In general, these bacterial infections were present in all but 30 of the 332 animals tested (90.4% prevalent), and were not concentrated in any particular focal host species (χ^2=9.7, *df*=6, *p*=0.139). Prevalence of each pathogen in each focal host species is presented in Figure 1.

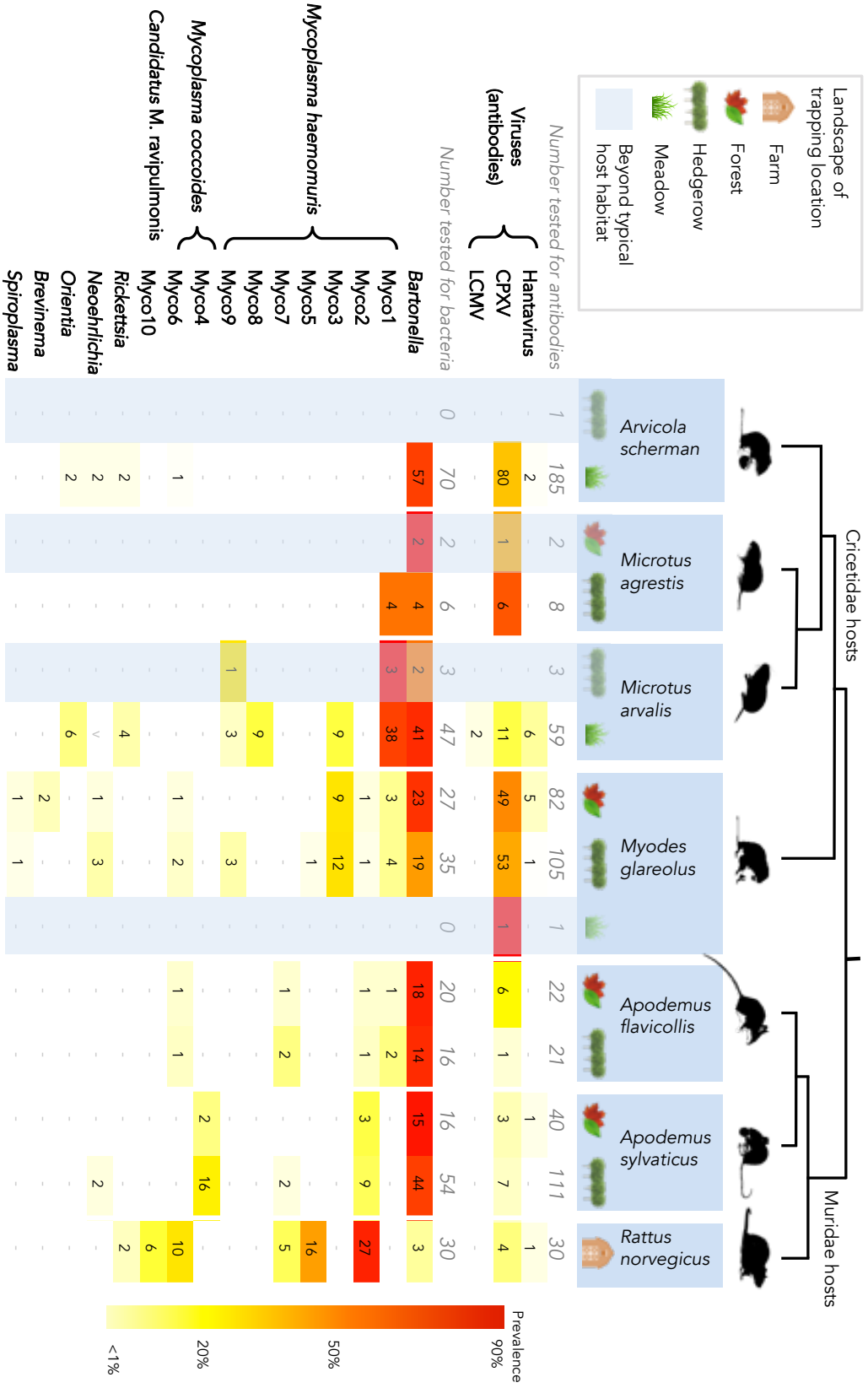


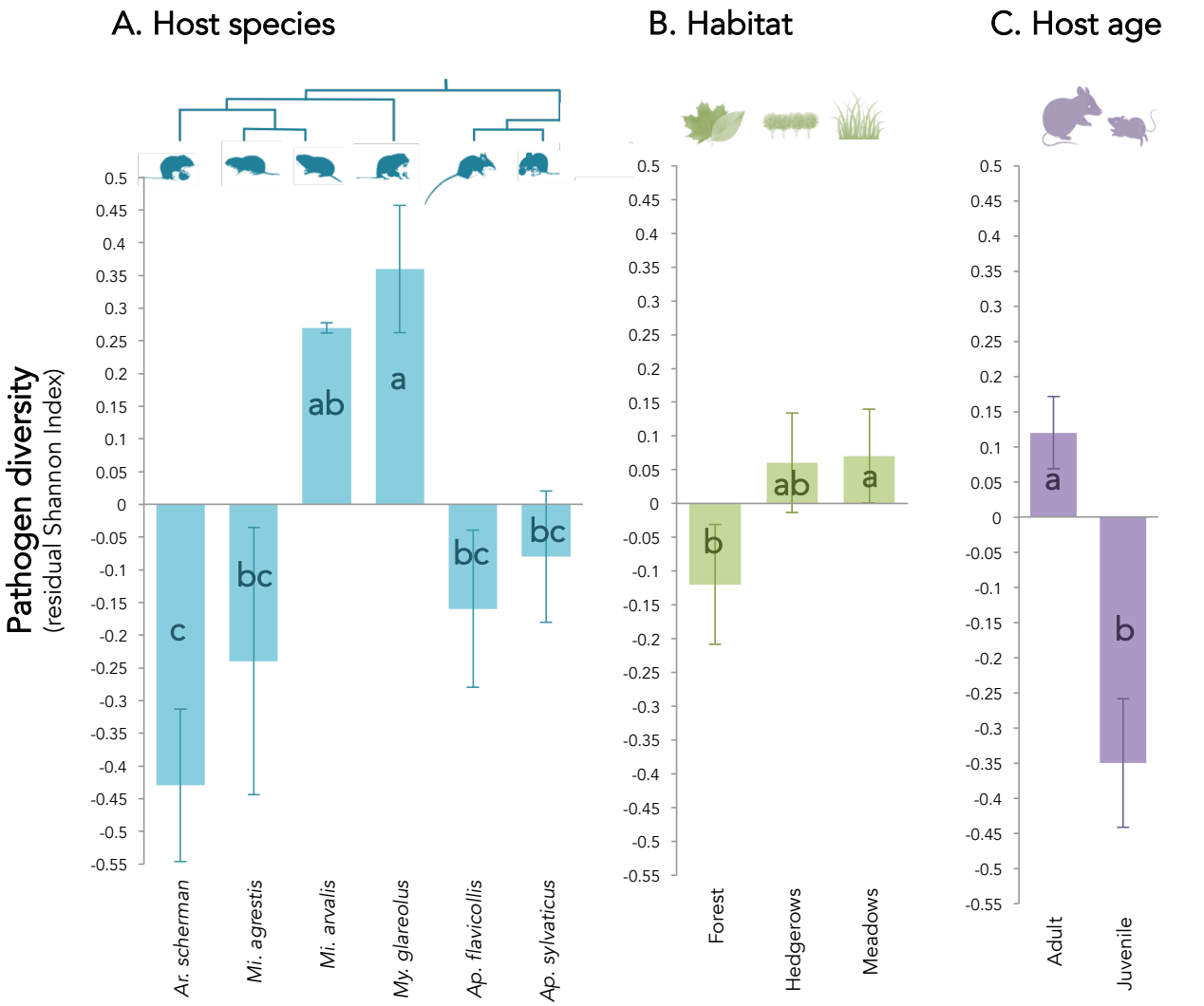
Figure 1. Pathogen occurrence across the rodent species community.

3.2. Extrinsic drivers of pathogen community diversity and composition within rodent community

3.2.1. Analyses of pathogen diversity

We found evidence for the co-circulation of between 3 (in *Mi. agrestis*) and 12 (in *My. glareolus*) pathogen taxa per host species across the rodent community (Figure 1). Using multiple regression analysis on the Shannon diversity index, we found that pathogen diversity differed significantly between habitats ($F_{2,78} = 5.63$, $p = 0.005$) and host species ($F_{5,78} = 7.12$, $p < 10^{-4}$). It was significantly higher in adults than in juveniles ($F_{1,78} = 32.29$, $p < 10^{-7}$). It did not differ between study sites, years, or host sexes (Figure 2, Table S3). Post-hoc Tukey tests showed that after

Figure 2: Extrinsic drivers of pathogen diversity in a rodent species community. Significant differences in Shannon diversity index were tested on residual variance of the multiple regression model controlling for all other extrinsic factors. Different letters signify statistically significant differences at $p < 0.05$, with post-hoc Tukey adjustments for multi-level factors.



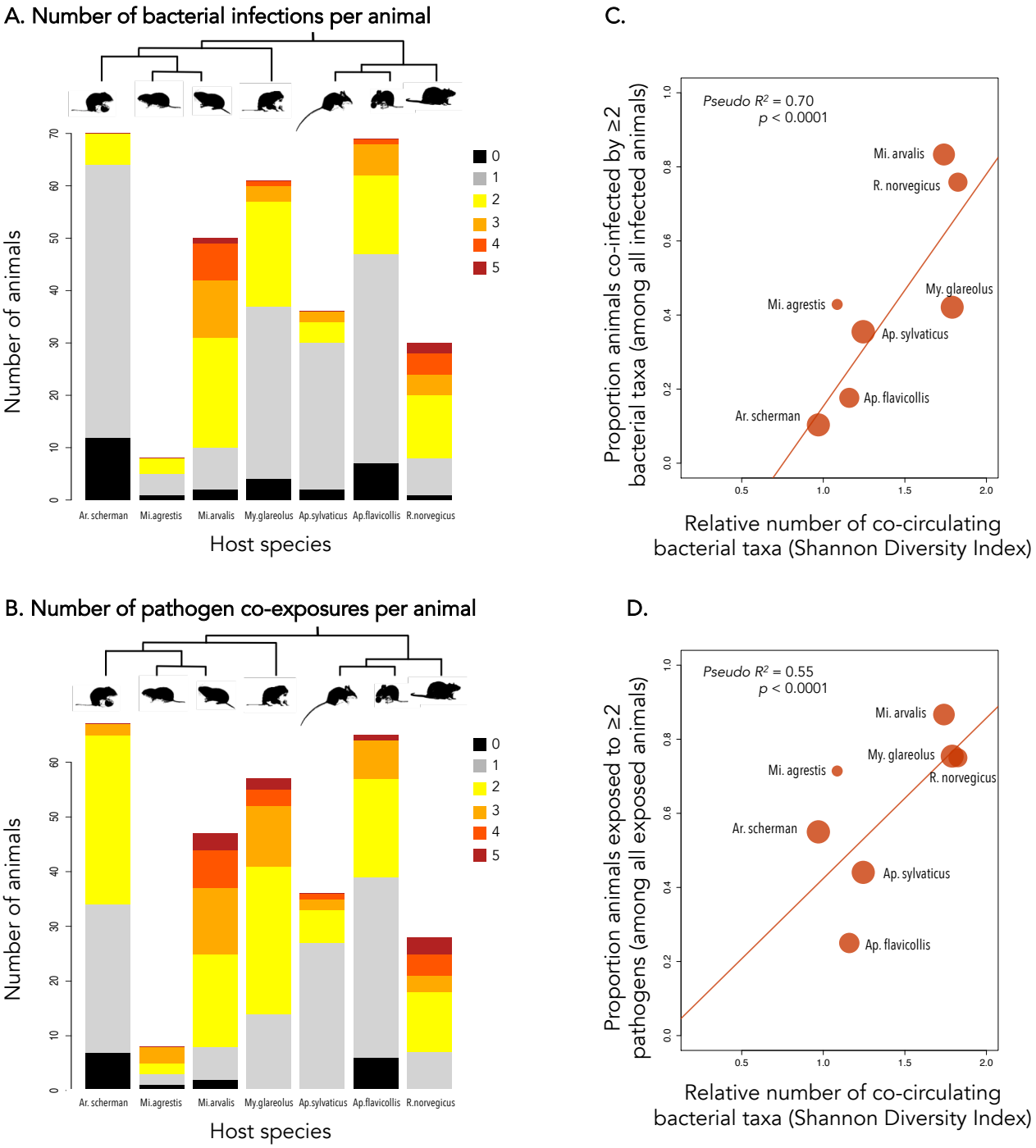
correcting for all other factors in the model, meadow habitats had higher diversity of pathogen exposure than forest habitats, and the diversity of pathogen communities in host species fell along a continuum between *My. glareolus* (high) and *Ar. scherman* (low) extremes (Figure 2, Table S3). To understand the relative pathogen diversity of *R. norvegicus* hosts, excluded from the model because they were entirely confounded with farm habitats, we analyzed two additional modified models : one excluding host habitat and the other excluding host species. Post-hoc Tukey tests from these models respectively showed that *R. norvegicus* hosts had the second most diverse pathogen community, and that farm habitats were thus in the same high-diversity category as meadow habitats (Table S4).

We also found an enormous amount of both bacterial co-infections and concurrent history of viral exposures (Figures 3A, 3B). The percentage of animals co-infected with two or more reliably detectable pathogenic bacterial OTUs among all those infected in each host species ranged between 84.4% (in *Mi. arvalis*) and 10.5% (in *Ar. scherman*). This co-infection frequency was significantly correlated with the diversity (Shannon Index) of bacteria circulating in each rodent species (Figure 3C; *Pseudo-*R^2 = 0.70, $p < 0.0001$, calculated using logistic regression weighted by the number of infected animals per species). Bacterial co-infections were more frequent than expected in *Mi. agrestis*, and less frequent than expected in *My. glareolus* (according to Cook's Distance, Figure S5A). Results were similar when co-occurrence of anti-viral antibodies were considered along with bacterial OTU exposure (Figure 3D; *Pseudo-*R^2 = 0.55, $p < 0.0001$). While *Mi. arvalis* had both more bacterial co-infections and slightly more pathogen co-exposures than expected based on pathogen diversity, other outliers differed between the two measures (Figure S5): *My. glareolus* co-exposure frequencies were not lower than expected, and both *Apodemus* species had lower than expected co-exposures.

3.2.2. Analyses of pathogen community composition

Many pathogen taxa were found only in a single host species (*Mycoplasma haemomuris* OTU Myco8, "*Candidatus* Mycoplasma ravipulmonis" (Myco10), *Brevinema* spp., *Spiroplasma* spp.,

Figure 3: Bacterial co-infection and co-exposure patterns across host species.



and LCMV), and each host species had a unique combination of co-circulating pathogens (Figure 1). In order to identify extrinsic factors (host species, host sex and age class, habitat, study site, year) potentially driving the composition of pathogen communities within the rodent community,

we performed a single MCA on occurrence data for exposure to both bacteria and viruses followed by logistic regression analyses. Due to competing *a priori* hypotheses that host species and habitat would be important factors, we excluded *R. norvegicus* individuals (as this host species was the only one found in farm habitats, confounding these two variables; but see MCA results when *R. norvegicus* was included in Figures S6, S7). Likewise, we excluded pathogens that occurred only in one habitat type of one host species (not including *R. norvegicus*). Two additional individuals were excluded due to missing sex and age information. Analyses were performed on the remaining 280 individuals from six host species and their 14 pathogens (*Bartonella*, Myco1, Myco2, Myco3, Myco4, Myco6, Myco7, Myco9, *Rickettsia*, *Neoehrlichia*, *Orientia*, *Spiroplasma*, and antibodies against CPXV and hantaviruses) (Figure S8).

Out of 14 orthogonal MCA dimensions returned, the first two captured 23.0% of the variation in pathogen and antibody occurrence, and the first seven explained a cumulative 63.4% of the total variance (Figure S9). Further dimensions captured less variance than would be expected if all dimensions contributed equally to overall inertia in the data. Dimension 1 (MCA Dim1; explaining 13.1% of the variation in pathogen community and loading heavily with the presence of Myco1, Myco3 and anti-hantavirus antibodies) differed significantly between host species ($F_{5,268} = 23.83$, $p < 0.0001$) and age classes ($F_{1,268} = 6.27$, $p = 0.013$; Table S5). Dimension 2 (MCA Dim2; explaining 9.9% of the variation in pathogen community and primarily describing the occurrence of *Bartonella*) was also structured significantly by host species ($F_{5,268} = 3.89$, $p = 0.002$; Table S5). While these first two dimensions varied by host species (Figure 4A) and host age class (Figure 4B), variance in host habitats (Figure 4C) was not significant after accounting for the other factors. Host species was the most consistently important extrinsic driver of pathogen community composition, significantly explaining variation captured in six of the first seven dimensions, MCA Dim1 – MCA Dim7 (except for MCA Dim 5; Table S5).

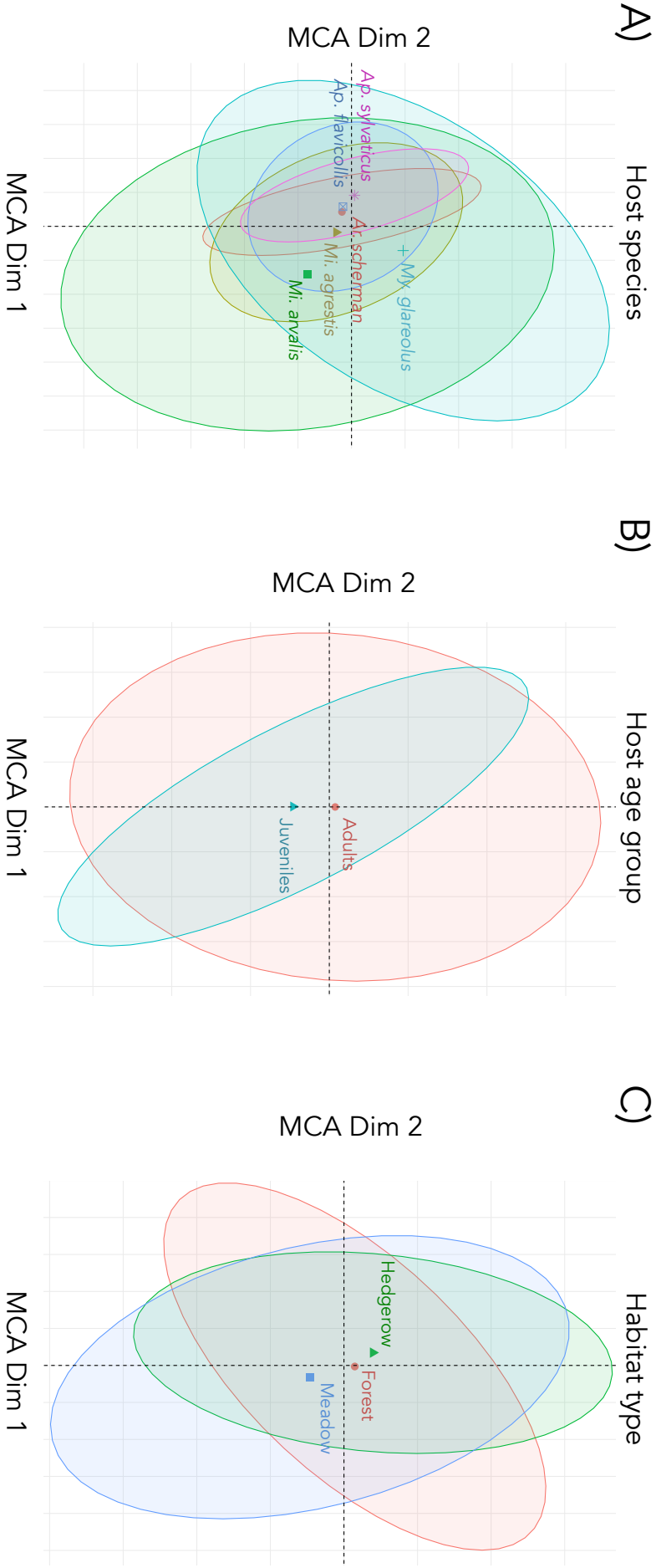


Figure 4. Pathogen community structure among extrinsic factors (A) host species (B) host age groups and (C) habitat types, represented by plotting the mean values for the first and second dimensions described by multiple correspondence analysis (MCA). MCA Dim1 and MCA Dim 2 collectively accounted for 23% of total variance in the data. Ellipses include 95% of individual values for each factor group.

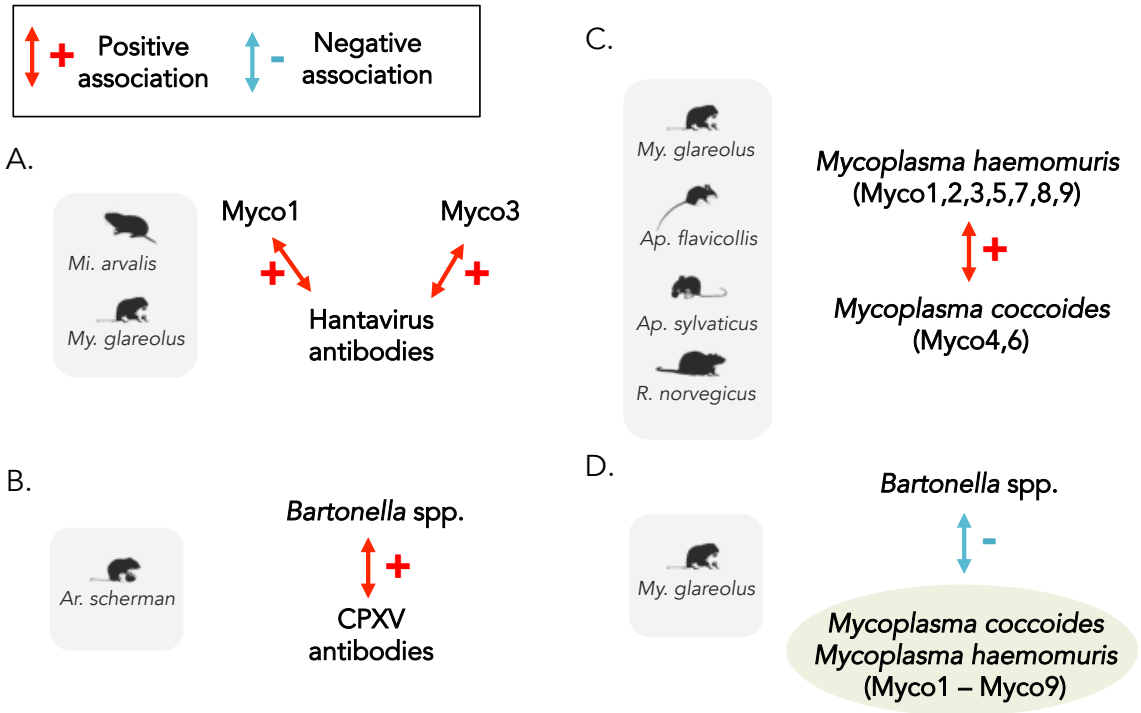
3.3. Associations between pathogens

3.3.1. Validation of the associations detected by MCA

We applied SCN and GLM analyses to further characterize patterns detected using MCA. Strong and relatively equal loading of MCA Dim1 with Myco1, Myco3, and anti-hantavirus antibody presence indicated that these three pathogens were positively associated with one-another. Indeed, the six animals with anti-hantavirus antibodies were found exclusively in animals infected with Myco3, and Myco1 was found in 2/3 of hantavirus-exposed animals but in just 1/3 of those without hantavirus exposure. The MCA also revealed significant differences among host species and host age classes for Dim 1; hantavirus and Myco 3 only circulated in two host species (*Mi. arvalis* and *My. glareolus*) and 34 of those 35 occurrences were in adults. To exclude positive associations arising from mutual host specificity and age-related accumulation of exposure probability, we focused our analyses on the dataset restricted to adults of the two host species in which all three pathogens co-circulated (*Mi. arvalis* and *My. glareolus*). Individual SCN analyses performed on adults of each host species revealed no associations (Table S6A). However, since values of MCA Dim 1 did not differ between the two host species (according to post-hoc tests given in Table S5), we also ran a single SCN analysis on the pooled data from adults of both species to improve statistical power (Table S6A). According to this pooled SCN analysis, the three-way co-occurrence of Myco1, Myco 3 and anti-hantavirus antibodies was significantly more frequent than would be expected by random chance ($p = 0.008$), with a trend for anti-hantavirus antibodies occurring by themselves more rarely than expected ($p = 0.13$; Table 6A, Figure S10). We also investigated this association using GLM. However given the small number of hantavirus exposures and perfect association with Myco3 infection, there was insufficient statistical power to explicitly test for an association between all three pathogens and extrinsic factors. We therefore ran three reciprocal GLM models on the restricted dataset, one for each pathogen as a function of extrinsic factors controlling for heterogeneous host groups (host species, host sex, study site, habitat, and year sampled) and exposure to the two other pathogens (Table S6B). These models showed that there

remained significant unexplained positive associations between hantavirus exposure and Myco1 infection (Hantavirus antibodies ~ Myco1: $\chi^2 = 5.67$, p = 0.017; Myco1 ~ Hantavirus antibodies: $\chi^2 = 2.89$, p = 0.09) and between hantavirus exposure and Myco3 infection (Myco3 ~ Hantavirus antibodies: $\chi^2 = 12.11$, $p < 0.001$), but that there was no evidence of direct association between Myco1 and Myco3 infections (Myco1 ~ Myco3: $\chi^2 = 0.07$, p = 0.8; Myco3 ~ Myco1: $\chi^2 < 0.01$, p = 0.96; Figure 5A).

Figure 5: Associations between pathogens in a community of rodents. Association hypotheses were generated by multiple correspondence analysis (A) or previously noted in the literature (B, C, D). Only associations supported by significant statistical tests ($p < 0.05$) are illustrated. Red arrows represent positive associations, blue arrows represent negative associations.



The third MCA dimension also presented a clear hypothesis with sufficient statistical power to be tested. MCA Dim3 was characterized by co-variation in Myco2 (*M. haemomuris*) and Myco4 (*M. coccoides*) infections suggesting a positive association between members of these two *Mycoplasma* species. Myco2 and Myco4 OTUs co-circulated only in *Ap. sylvaticus* hosts, thus we limited our analysis to this host species. There was no significant association between the two OTUs detected by SCN analysis (Table 7A), and after correcting for all extrinsic factors, there

446 remained only a non-significant trend (Myco2 ~ Myco4: χ^2 = 2.47, p = 0.12; Myco4 ~ Myco2: χ^2 =
447 2.47, p = 0.12; Table S7B) for a positive association between the two OTUs. No additional
448 associations with sufficient variance for statistical tests were clearly suggested by the MCA
449 analysis.
450
451 *3.3.2. Validation of associations described in the literature*
452 We tested the *a priori* hypothesis that seropositivity to CPXV would be positively associated
453 with *Bartonella* infection, previously detected in *Mi. agrestis* (Telfer *et al.* 2010). The whole dataset
454 was considered as these two pathogens co-circulated in all host species (Figure 1). SCN analyses
455 performed independently for each host species revealed no associations (Table S8A), and the MCA
456 results suggested that pooling data across host species would be inappropriate. After correcting for
457 extrinsic factors using GLM, we found reciprocal evidence for a positive association in *Ar.*
458 *scherman* hosts (*Bartonella* ~ CPXV antibodies: χ^2 = 5.37, p = 0.020; CPXV antibodies: χ^2 = 5.21,
459 p = 0.022; Figure 5B), but not in any other host species (Table S8B). It is of note that there were
460 only eight *Mi. agrestis* individuals, rendering statistical power to test for the association while
461 controlling for extrinsic factors insufficient in this host species where the association was
462 previously described. While prevalence of both pathogens in *Mi. agrestis* was relatively high
463 compared to other host species, one of the two animals without *Bartonella* infection was positive
464 for CPXV antibodies, also precluding evidence for a within-species trend.
465 We next focused on the potential associations between OTUs identified as belonging to two
466 different species of hemotropic *Mycoplasma, M. haemomuris* (HM) and *M. coccoides* (HC), within
467 the four host species in which they both circulated (*My. glareolus*, *Ap. flavicollis*, *Ap. sylvaticus*, *R.*
468 *norvegicus*; Figure 1; Figure S4). We found no significant associations using independent SCN
469 analyses for each host species (Table S9A). However, after controlling for extrinsic factors using
470 GLM, a significant positive association was detected (HM ~ HC: χ^2 = 10.27, p = 0.0013; HC ~ HM:
471 χ^2 = 9.92, p = 0.0016), and did not differ between host species (non-significant interaction term

between host species by explanatory pathogen occurrence in each reciprocal model, Table S9B; Figure 5C). We note that only one *R. norvegicus* animal was uninfected with *M. haemomuris*, and that animal also had no *M. coccoides* infection; thus the trend for the association in this host species was also positive but lacked sufficient variance for independent statistical analysis.

Finally, we tested for associations between *Bartonella* spp. and hemotropic *Mycoplasma* species, grouping the occurrence of different OTUs of the latter (Myco1 – Myco9) into a single presence-absence variable. There was no association detected by SCN analyses (Table S10A), and marginal evidence that any association may differ by host species after correcting for extrinsic factors using GLM (Table S10B). After controlling for extrinsic factors using independent GLMs for each host (where possible), we found a negative association between the two pathogen groups only in *My. glareolus* hosts (*Bartonella* ~ *Mycoplasma*: $\chi^2 = 5.73$, $p = 0.017$; *Mycoplasma* ~ *Bartonella*: $\chi^2 = 5.65$, $p = 0.017$, Table S10B, Figure 5D).

4. Discussion

Rodents have long been recognized as important reservoirs of infectious agents, with a high transmission potential to humans and domestic animals. Europe is identified as a hotspot of rodent reservoir diversity and one third of rodent species are considered hyper-reservoirs, carrying up to 11 zoonotic agents (Han *et al.* 2015). Nevertheless, associations between these pathogens have still only rarely been investigated (but see, for example, studies from field voles in the UK (Telfer *et al.* 2010) and in Poland (Pawelczyk *et al.* 2004), gerbils in Israel (Cohen *et al.* 2015), across a rodent community in North America (Dallas *et al.* 2019), and co-infection frequencies of zoonotic pathogens from rodents in Croatia (Tadin *et al.* 2012)).

In this study, we confirmed that rodent communities in northern France may harbor a large diversity of potential zoonotic pathogens, with at least 10 bacterial genera (23 OTUs) and antibodies against four genera of viruses circulating in the areas examined. Some of these pathogens have already been reported in this region or in geographic proximity, including viruses

(*Orthohantavirus*, *Orthopoxvirus*, *Mammarenavirus* (Charbonnel *et al.* 2008; Salvador *et al.* 2011)); and bacteria (e.g. *Bartonella, Mycoplasma, Rickettsia, "Candidatus* Neoehrlichia*", Orientia, Spiroplasma, Treponema, Leptospira, Borrelia, Neisseria, Pasteurella*; see (Vayssier-Taussat *et al.* 2012; Razzauti *et al.* 2015)). Several previously unseen agents were also detected, such as Ljungan virus, a picornavirus found in other European rodent populations (e.g., Salisbury *et al.* 2014), and a relative of the putatively pathogenic spirochaete *Brevinema andersonii* that infects short-tailed shrews and white-footed mice in North America (Defosse *et al.* 1995). In addition, we detected a relatively high prevalence of anti-hantavirus antibodies in *Mi. arvalis*, explained by cross-reactivity between the anti-PUUV antibodies used in our assay and those elicited against the related *Tula orthohantavirus* (TULA) virus common to European voles (Deter *et al.* 2007; Tegshduuren *et al.* 2010).

Three pathogens were highly prevalent. Anti-*Orthopoxvirus* antibodies were detected in all seven focal rodent species, with prevalence levels reaching up to 70%. This corroborates prior evidence that cowpox virus could be widespread in European rodents, particularly voles (Bennett *et al.* 1997; Essbauer, Pfeffer & Meyer 2010; Forbes *et al.* 2014). An astounding 77% of all individuals in the study were infected by *Bartonella sp.*, with *R. norvegicus* being the notable exception (with only 10% infected, leaving an average of 84% prevalence in all other species). The bacterial genus *Bartonella* is a diverse group of hemotrophs known to commonly infect rodents and other mammals (Breitschwerdt & Kordick 2000; Bai *et al.* 2009) and which have also been implicated in both zoonotic and human-specific disease (Iralu *et al.* 2006; Breitschwerdt 2014; Vayssier-Taussat *et al.* 2016). We could not assess the specific diversity of *Bartonella* sp. circulating in these rodent communities because accurate resolution in this genus requires additional genetic markers (Matar *et al.* 1999; Guy *et al.* 2013). Hemotropic and pneumotropic *Mycoplasma* spp. were also highly prevalent, collectively infecting 43% of all hosts, with nearly all *R. norvegicus* and *Mi. arvalis* samples infected. On the contrary, we found just one *Mycoplasma* infection in *Ar. scherman,* despite ample sampling (70 animals tested) and its previous detection in

524 *A. scherman* from Eastern France (Villette *et al.* 2017). These *Mycoplasma* species are also known
525 pathogens of humans and rodents (Harwick, Kalmanson & Guze 1972; Baker 1998). Here, we
526 found 15 different OTUs corresponding to two distinct hemotropic *Mycoplasma* species (*M.*
527 *haemomuris* and *M. coccoides*) and the pneumotropic *Mycoplasma* species *M. pulmonis* and
528 "*Candidatus* M. ravipulmonis". The former two are both hemotropic mycoplasmas responsible for
529 vector-transmitted infectious anaemia of wild mice, rats, and other rodent species (Neimark *et al.*
530 2001, 2005; Messick 2004). However, *M. pulmonis* and "*Candidatus* M. ravipulmonis" cause
531 respiratory infections, are more closely related to other pneumotropic mycoplasmas, and
532 "*Candidatus* M. ravipulmonis" has only ever before been described as spontaneously occurring in
533 laboratory mice and rats exposed to unknown microbes of other animals and humans via passaging
534 experiments (formerly termed Grey Lung virus; (Andrews & Glover 1945; Neimark, Mitchelmore
535 & Leach 1998; Graham & Schoeb 2011; Piasecki, Chrzastek & Kasprzykowska 2017)).

536 Lastly, our data corroborated the status of hyper-reservoir (more than two zoonotic pathogens
537 carried by a reservoir species) for all seven of the focal rodent species studied here (Han *et al.*
538 2015), with the addition of *Mi. subterraneous* that we also found to carry two potentially zoonotic
539 pathogens (*Bartonella* spp. and *Brevinema* spp.; Appendix 1). Overall, we found a high variability
540 in the number of pathogens circulating in each species despite correction for sampling effort, with
541 low levels observed in *Apodemus* species and *Arvicola scherman*, and high levels detected in *Mi.*
542 *arvalis, My. glareolus*, and *R. norvegicus*.

543 The search for factors that drive parasite species richness, diversity and community composition
544 has been at the core of numerous studies (Poulin 1995; Poulin & Morand 2000; Nunn *et al.* 2003;
545 Mouillot *et al.* 2005; Krasnov *et al.* 2010). Here, we emphasized that both pathogen diversity and
546 community composition was mainly structured by host species identity, despite both shared habitats
547 and shared pathogen taxa. Using multiple correspondence analysis (MCA) together with logistic
548 regression and the novel computational method of association screening (SCN) analysis, we found
549 no evidence that any specific pathogen-pathogen associations were likely to be as important as host

550 species identity in determining pathogen distributions across the community of rodents. The strong
551 influence of host characteristics (Cohen *et al.* 2015) and host species identity (Dallas *et al.* 2019) on
552 pathogen community composition has recently been described in comparison to intrinsic pathogen-
553 pathogen associations in other rodents. Moreover, the pathogen community composition provided a
554 unique signature of each rodent species, even among those most closely related (e.g. *Ap. flavicolis*
555 and *Ap. sylvaticus*). This result is in line with the conclusions of recent meta-analyses showing that
556 phylogeny, over other host traits, had a minimal impact on pathogen diversity in rodent species
557 (Luis *et al.* 2013; Guy *et al.* 2019).

558 The importance of host species identity in shaping pathogen community composition may not
559 stem from strict host-pathogen specificity, as most pathogens were found to infect multiple host
560 species – a broad result echoed across animal communities (Taylor *et al.* 2001; Woolhouse, Taylor
561 & Haydon 2001; Cleaveland, Laurenson & Taylor 2001; Pedersen *et al.* 2005; Streicker *et al.*
562 2013). However, we might be cautious as more precise molecular analyses are necessary to test
563 whether different species of a bacteria genus or divergent populations of the same bacteria species
564 may circulate independently in different rodent host species, with little or no transmission. For
565 example, two genera seemed to be largely shared among the rodent species studied here, *Bartonella*
566 and *Mycoplasma*. But previous studies have shown strong host-specificity when considering the
567 genetic variants of *Bartonella* (Buffet *et al.* 2013; Withenshaw *et al.* 2016; Brook *et al.* 2017)
568 Evidence in the literature for host specificity of *Mycoplasma* species has led to a mix of conclusions
569 (Pitcher & Nicholas 2005), as cases of cross-species transmission are commonly reported –
570 particularly in humans – despite a general consensus that most species are highly host-specific. We
571 found that some *Mycoplasma* taxa were dominant contributors to prevalence in a single host
572 species, and that when shared, they were shared with just a few other specific host species. Rare
573 infections in unexpected host species (e.g., Myco6 in *Ar. scherman* and Myco1 in *Ap. flavicollis*)
574 were represented by fewer sequence reads compared to positive samples in host species where they
575 were more prevalent, suggesting a low potential for amplification and sustained transmission from

these occasional hosts (Figure S4). On the other hand, while the Cricetidae appeared to be susceptible only – with rare exception – to taxa within the *M. haemomuris* group, host species in the Muridae family were susceptible to all three distinct *Mycoplasma* species detected, The biggest exception to this pattern was that three of 62 *Myodes glareolus* (the most basal member of the Cricetidae included in the study) animals were found to be infected by both hemotropic *Mycoplasma* species. These results both support the observation that cross-species transmission naturally occurs among wild rodents and suggest that the degree of host specificity may be driven by both host and pathogen factors.

Several studies have emphasized the influence of host habitat specialization on parasite species richness, low habitat specialization being associated with both high species richness of macro- and micro-parasites (e.g. (Morand & Bordes 2015)). Our results did not fully corroborate this association; while the grassland-specific *Ar. scherman* had the lowest pathogen diversity and the multi-habitat spanning *My. glareolus* had the highest pathogen diversity, entirely farm-dwelling *R. norvegicus* had high pathogen diversity nearly equal to that of *My. glareolus*, and the two *Apodemus* hosts (neither with significantly higher pathogen diversity than *Ar. scherman*) were found across both meadows and hedgerows. We also found no influence of sampling sites or years on pathogen diversity at the small spatio-temporal scale considered here. Hence, further research is required to decipher the eco-epidemiological features that explain this strong influence of rodent species on pathogen community composition, such as host densities, home ranges, behaviours, or genetics (e.g. (Morand 2015).

We found that a large proportion of rodents from Northern France were co-infected with two or more bacterial pathogens (max = 5 bacterial pathogens in *Mi. arvalis* and *R. norvegicus* hosts), and also had concurrent histories of exposure to multiple viruses (max = 5 bacterial and/or viral co-exposures in *Mi. arvalis*, *My. glareolus*, *Ap. sylvaticus*, and *R. norvegicus* hosts). The percentage of pathogen co-exposed hosts was as high as 89 % (in *Mi. arvalis* hosts), dropping only to 83% when considering only presently co-infecting bacterial taxa. These results are in line with recent studies

602 that have shown that co-infections by multiple pathogens are common in natural populations (e.g. in
603 mammals, birds, amphibians, ticks, humans (Telfer *et al.* 2010; Griffiths *et al.* 2011; Moutailler *et*
604 *al.* 2016; Clark *et al.* 2016; Stutz *et al.* 2018)). Here, we also observed high variability in the
605 percentage of individuals with evidence of multiple current or prior infections between the rodent
606 species investigated. This variation in pathogen co-exposure was highly correlated to the diversity
607 of pathogens circulating in each host species, suggesting the dominance of a random process of
608 pathogen exposure for each individual. However, there were a few intriguing outliers: *My. glareolus*
609 hosts were less co-infected than expected based on diversity of bacterial taxa, but not when viral
610 antibodies were included; conversely, *Ar. scherman* hosts were more co-exposed when viruses were
611 considered, but not when only bacteria were considered; and *M. arvalis* hosts had consistently
612 higher proportions of co-exposures whether viruses were or were not considered along with
613 bacteria. The non-random grouping of pathogen exposures within individuals (as in *M. arvalis*) may
614 result from heterogeneity in extrinsic transmission, environmental, or susceptibility factors
615 (Cattadori *et al.* 2006; Swanson *et al.* 2006; Beldomenico *et al.* 2008; Fenton, Viney & Lello 2010;
616 Beldomenico & Begon 2010) or from intrinsic interactions between pathogens (e.g. facilitation
617 mediated by hosts immune response). Differences in the pattern of co-exposure frequencies when
618 including or excluding antiviral antibodies (as with *M. glareolus* and *Ar. scherman*) could result
619 from different mechanisms (e.g., bacterial manipulation of innate immunity (Diacovich & Gorvel
620 2010)) affecting pathogen community assemblage. However, a lack of deviance from the expected
621 co-exposure frequency does not exclude the possibility that both extrinsic and intrinsic processes
622 may be occurring.

623 Here, we tested for pathogen-pathogen associations suggested by multiple correspondence
624 analysis (MCA) of the structure of pathogen exposures in this specific rodent community, as well as
625 those previously described in the literature. We used multiple logistic regression analyses (via
626 analysis of deviance on generalized linear models (GLMs)) to determine whether pathogen-
627 pathogen associations were present after extrinsic factors creating heterogeneity among hosts were

taken into account. We found evidence in support of three previously identified associations (positive association between *M. haemomuris* and *M. coccoides* infections; positive association between *Bartonella* infection and the presence of CPXV antibodies; negative association between *Bartonella* and hemotropic *Mycoplasma* infections), and characterized one set of associations not previously described (positive associations between the presence of hantavirus antibodies and infections by two specific *M. haemomuris* OTUs) – each in a unique subset of host species.

The positive association between *Mycoplasma haemomuris* and *M. coccoides* was found in the four rodent species where these bacteria co-circulate (i.e., *My. glareolus, Ap. flavicollis*, *Ap. sylvaticus* and *R. norvegicus*). Transmission of *Mycoplasma* blood infections is not yet well understood, but given a lack of support for density-dependent transmission and high incidence of spill-over events, it is likely that these bacteria are transmitted through bites of blood-sucking arthropod vectors (Volokhov *et al.* 2017). The positive associations detected between *M. haemomuris* and *M. coccoides* could also result from similarities in rodent susceptibility. Indeed *Mycoplasma* infection can lead to acute or chronic infection, and the establishment of chronic bacteremia seems to occur in immunosuppressed or immunocompromised individuals (Cohen *et al.* 2018). Co-infections with multiple *Mycoplasma* spp. might therefore be more likely to be detected in these immunocompromised rodents with chronic infections. The existence of chronic infections might also lead to additional co-infections and positive associations as a result of disease-induced changes in population dynamics, immune system function, or through direct pathogen-pathogen interactions (Fenton 2008; Aivelo & Norberg 2018; Fountain-Jones *et al.* 2019).

Whether through the accumulation of exposure probabilities or increased susceptibility, the previously-undocumented positive association we found here between *M. haemomuris* OTUs (Myco1 and Myco3) and hantavirus antibodies may similarly be explained by the chronic nature of both *Mycoplasma* and hantavirus infections in rodents (e.g. for Puumala hantavirus in bank voles (Yanagihara, Amyx & Gajdusek 1985; Meyer & Schmaljohn 2000; Vaheri *et al.* 2013)). This positive association was found in both host species where the majority of hantavirus exposures

654 occurred (*Microtus arvalis* and *Myodes glareolus*), consistent with the generality of association
655 between *Mycoplasma* species across host taxa detailed above, suggesting the intrinsic ecology of
656 these pathogens contributes to shaping variation in the pathogen community independent of host
657 species identity. Curiously, we found no evidence for direct associations between OTUs of the same
658 *Mycoplasma* species, thus facilitation interactions are unlikely to explain the high diversity of
659 *Mycoplasma* taxa both within and between host species.

660 Infections by *Bartonella* species are also known to often result in subclinical and persistent
661 bacteremia in mammals, including rodents (Birtles *et al.* 2001; Kosoy *et al.* 2004). The positive
662 association detected in *Ar. scherman* between *Bartonella* spp. and cowpox virus antibodies might
663 therefore be explained by, for example, joint accumulation of both chronic bacterial infections and
664 long-lived antibodies used to test for prior exposure to relatively short-lived CPXV virus infections.
665 However, if the same processes governing association of the chronic infections described above
666 were at play here, we would have expected to find both pathogens implicated in positive
667 associations (i) with other chronic infections, and (ii) across host species given their ubiquitous
668 prevalence. While the failure to recover the association in *Mi. agrestis* (where it was previously
669 described (Telfer *et al.* 2010) was likely due to low statistical power, the lack of a general pattern
670 across other host species despite adequate sampling suggests a more specific, and potentially
671 immune-mediated, ecological process between these two pathogens. Indeed, pox virus infections,
672 including CPXV, have been shown to induce immunomodulation that increases host susceptibility
673 to other parasites (Johnston & McFadden 2003). These interactions could be of variable intensities
674 according to the rodent species considered, due to potential differences in impacts of CPXV
675 infection on immunity across host species, or to the influence of other infections not examined here
676 on host immune responses during pox infections (e.g. helminths (Cattadori, Albert & Boag 2007),
677 protozoa (Telfer *et al.* 2010)). Furthermore, *Bartonella* infection was negatively associated with
678 *Mycoplasma* infections in *My. glareolus*, corroborating negative interactions revealed in co-
679 infection experiments in gerbils (Eidelman *et al.* 2019). This association may therefore originate

680 from an interaction mediated by specific (immune-) genetic features of *My. glareolus*, and not
681 ecological conditions as proposed by Eidelman et al. (2019). The antagonistic and host-specific
682 nature of this association lends further support to the interpretation that *Bartonella* infections do not
683 behave in similar ways to other chronic infections in the community. However, few studies have
684 investigated the robustness of within-host interactions across different host species (e.g., (Lello *et*
685 *al.* 2018)), and this question deserves further investigation.

686 Our results suggest that intrinsic ecological interactions could help shape the composition of the
687 pathogen community within hosts. However, this suggestion provides only a hypothesis that
688 requires further investigation to establish its provenance. Interpretation of associations can be
689 misleading, as they may arise from unmeasured co-factors such as exposure to shared transmission
690 routes, and may even run counter to the underlying ecological process (Fenton *et al.* 2014). We
691 noted that the associations we found here were considered properly characterized only once
692 extrinsic factors had been taken into account using multiple logistic regression analyses, and were
693 not visible (or even misleading, in the case of a 3-way interaction between hantavirus, Myco1 and
694 Myco3) when ignoring these factors using the SCN analysis, despite the increased statistical power
695 it offered. Evidence for interactions between pathogens within hosts initially came from laboratory
696 studies (e.g. in the development of vaccines, reviewed in (Casadevall & Pirofski 2000)), and until
697 recently, many studies conducted in the wild could not detect such interactions (e.g. (Behnke
698 2008)). Developments of statistical approaches have contributed to improve sampling designs and
699 analyses, in particular by better controlling for confounding factors, enabling the detection of
700 associations resulting from these within-host interactions (e.g., (Lello *et al.* 2004; Telfer *et al.*
701 2010)). Experiments conducted in semi-controlled environments have been used to confirm the
702 importance of interactions suggested by the associations (e.g. (Knowles *et al.* 2013)). Both
703 facilitation mediated by immune responses (e.g., (Ezenwa *et al.* 2010)) and competition mediated
704 by shared resources (e.g., (Brown 1986; Budischak *et al.* 2018)) have been emphasized.

There remain additional important limits to the interpretation of snapshot observational studies from wild populations such as we have presented here. For instance, they can not provide information about the sequence and timing of infection, although these features strongly affect the outcome of within-host interactions (Eidelman *et al.* 2019). Moreover, both the 16S metabarcoding approach and serological antibody tests can only be interpreted in terms of presence/absence of exposure to pathogens, although co-infection may rather impact parasite abundance (e.g., (Thumbi *et al.* 2013; Gorsich, Ezenwa & Jolles 2014)). Lastly, we also acknowledge several caveats to consider with our methods. We removed animals from which fewer than 500 reads were amplified in one or both bacterial metabarcoding PCR replicates. While 16 of these samples removed were due to random failure of PCR amplification from just one of the two replicates, 12 of the animals had poor amplification in both PCR replicates. In the absence of an internal positive control, e.g. a spike-in standard (Zemb *et al.* 2020), we were unable to verify whether a lack of reads was due to poor DNA extraction or a true lack of infections. Although this has a risk of artificially inflating prevalence rates by selectively removing uninfected individuals, it is unlikely to have had a qualitative effect on our results. Similarly, limiting our analyses to OTUs with 500 reads or more in the entire dataset may select against detection of very rare or low-burden infections. We also removed many OTUs corresponding to bacteria normally occurring in external or internal microbiomes of healthy animals, some of which were represented by a high abundance of reads in positive animals. We know that, for instance, *Helicobacter* species are naturally found in the digestive tract, but can also cause pathogenic infections. We also know that parasitism can affect host microbiome composition (Gaulke *et al.* 2019), and this in turn can have impacts on host health and disease susceptibility (reviewed in (Murall *et al.* 2017)). Thus, our choice to ignore OTUs corresponding to microbes typical of healthy flora contributes to the problem of missing data, such as information on intestinal helminth infections, which may explain or alter the associations we were able to detect. These caveats are common problems for disease surveillance and community ecology studies, irrespective of the diagnostic methods, and it is difficult to speculate about their

overall impacts on the present study. Finally, it is well-understood that this bias towards detection of common pathogens and difficulty in interpreting evidence for the absence of a pathogen in a given individual or population can make testing for negative associations driven by antagonistic ecological interactions incredibly difficult, if not impossible (Weiss *et al.* 2016; Cougoul *et al.* 2019).

Conclusions

Our results add to a growing number of studies finding that (i) rodents host many important zoonotic human pathogens and (ii) pathogen communities are shaped primarily by host species identity. We also detected a number of previously un-described associations among pathogens within these rodent communities, and we also confirmed previously identified associations, sometimes in other rodent species than those in which they were previously described. These associations can be considered in the future as hypotheses for pathogen-pathogen interactions within rodents hosts, and that participate in shaping the community of pathogens in rodent communities. Long-term survey and experimental studies are now required to confirm these interactions and understand the mechanisms underlying the patterns of co-infection detected. In addition to these biological results, we have identified several methodological caveats, with regard to both pathogen and association detection, that deserves further investigation to improve our ability to make robust inference of pathogen interactions.

Acknowledgements

We would like to thank all collaborators and research assistants that previously helped with the field work (Yannick Chaval, Cécile Gotteland, Marie-Lazarine Poulle). This study was partly funded by the INRA Metaprogram MEM Hantagulumic, and by the European programme FP7-261504 EDENext. The manuscript is registered with the EDENext Steering Committee as EDENext409.

None of the rodent species investigated here has protected status (see list of the International Union for Conservation of Nature). All procedures and methods were carried out in accordance with relevant regulations and official guidelines from the American Society of Mammalogists. All protocols presented here were realized with prior explicit agreement from relevant institutional committee (Centre de Biologie pour la Gestion des Populations (CBGP): 34 169 003).

We thank Hélène Vignes of the Cirad Genotyping platform for her assistance with the MiSeq sequencing, and Sylvain Piry, Alexandre Dehne-Garcia and Marie Pagès for their help with the bioinformatic analysis.

Data Accessibility

Supplementary data deposited in Dryad (https://doi.org/XXXXXXXXXXX) include the following 16S metabarcoding data: (i) raw sequence reads (fastq format), (ii) raw output files generated by the mothur program (iii) raw abundance table and (iv) filtered occurrence table.

771 Table 1. BLAST search results for OTUs suspected of belonging to pathogenic genera.
772

Infecting species identity	OTU Number	Nuimber of Reads	Genbank Accession Number	BLAST results (% identity)	Pathogen Code
Pathogenic taxa, reliably detectable					
Bartonella spp.	Otu00001	6353372	MT027154	100% *Bartonella grahamii* (AB426637) from wild North America rodents; 99%-100% identity to many other pathogenic *Bartonella* species.	Bartonella
Brevinema spp.	Otu00123	5603	MT027155	97% *Brevinema andersonii* (NR_104855) type sequence, infectious spirochaete of short-tailed shrew and white-footed mouse in North America	Brevinema
Candidatus Neoehrlichia mikurensis	Otu00039	18358	MT027156	100% *Candidatus* Neoehrlichia mikurensis (KF155504) tick-borne rodent disease, opportunitstic in humans	Neoehrlichia
Mycoplasma ravipulmonis	Otu00054	6086	MT027164	100% Mycoplasma ravipulmonis (AF001173), grey lung disease agent in Mus musculus; 88% *M. orale* from humans (LR214940)	Myco10
Mycoplasma spp.	Otu00004	845971	MT027157	99% identity to uncultured *Mycoplasma* species (KU697344) from small rodents in Senegal and uncultured eubacterium (AJ292461) from Ixodes ticks; 95% (KM538694) and 94% (MK353834) identity to uncultured hemotropic *Mycoplasma* species in European and South American bats	Myco1
Mycoplasma spp.	Otu00003	426034	MT027158	99% *Mycoplasma haemomuris* (AB758439) from *Rattus rattus*	Myco2
Mycoplasma spp.	Otu00006	106443	MT027159	99% uncultured *Mycoplasma* spp. (KP843892) from leopards in South Korea; 99% identity to uncultured *Mycoplasma* (KT215637) from rodents in Brazil	Myco3
Mycoplasma spp.	Otu00010	72724	MT027160	99% *Mycoplasma coccoides* comb. nov. (AY171918); 97% *Candidatus* Mycoplasma turicensis (KJ530704) from Indian mongoose	Myco4
Mycoplasma spp.	Otu00005	165095	MT027161	100% *Mycoplasma haemomuris*-like undescribed species (KJ739312) from *Rattus norvegicus*	Myco5
Mycoplasma spp.	Otu00007	92237	MT027162	99% uncultured *Mycoplasma* spp. (KC863983) from *Micromys minutus* (eurasian harvest mouse) in Hungary; 98% *M. coccoides* comb. nov.(AY171918)	Myco6
Mycoplasma spp.	Otu00012	39767	MT027163	93% uncultured *Mycoplasma* species (KU697341) of mice in Senegal; 92% *Mycoplasma* spp. (KP843892) from leopards in South Korea, 91% *Mycoplasma haemomuris* (AB820289) in rats	Myco7
Mycoplasma spp.	Otu00015	31528	MT027165	98% uncultured *Mycoplasma* spp. (KT215632) from wild rodent spleen in Brazil; 95% uncultured *Mycoplasma* spp. (KF713538) in little brown bats	Myco8
Mycoplasma spp.	Otu00049	40125	MT027166	96% uncultured *Mycoplasma* spp. from Brazilian rodents (KT215638) and S. Korean leopard (KP843892)	Myco9
Orientia spp.	Otu00111	876	MT027167	97% *Orientia tsutsugamushi* (KY583502) from humans in India, zoonotic Rickettsial pathogen (causes scrub typhus)	Orientia
Rickettsia spp.	Otu00008	72098	MT027168	98% *Rickettsia japonica* (MF496166) which causes Japanese spotted fever, *R. canadensis* (NR_029155) & *R. rhipicephali* (NR_074473) type strains	Rickettsia
Spiroplasma spp.	Otu00093	4738	MT027169	95% uncultured *Spirolasma* spp. (KT983901) from Ixodes tick on a dog; 94% identity to type strain of *Spiroplasma mirum* (NR_121794), the agent of suckling mouse cataract disease; *Spiroplasma ixodetis* causes similar disease in humans.	Spiroplasma
Pathogenic taxa, not reliably detectable					
Arcobacter cryaerophilus	Otu00296	403	MT027170	100% *Arcobacter cryaerophilus* (CP032825) emerging enteropathogen in humans, zoonotic, pathogenic in rats	Arcobacter
Borrelia miyamotoi	Otu00318	419	MT027171	100% *Borrelia miyamotoi* (CP010308)in humans and Ixodes, zoonotic pathogen	Borrelia1
Borrelia spp.	Otu00514	206	MT027172	96% *Borrelia* sp. nov "Lake Gaillard" in *Peromyscus leucopus* (AY536513), 95% *B. hermsii* (MF066892) from tick (*Ornithodoros hermsi*) bites in humans	Borrelia2
Borrelia afzelii	Otu00071	78	MT027173	100% *Borrelia afzelii* (CP009058) human pathogen closely related (98%) to *B burgdorferii* (positive control sequence)	Borrelia3
Leptospira spp.	Otu01015	257	MT027174	100% several pathogenic *Leptospira* species from mammals, e.g., *L. interrogans* (LC474514) in humans	Leptospira
Mycoplasma pulmonis	Otu00771	255	MT027175	100% *Mycoplasma pulmonis* (NR_041744) chronic respiratory pathogen of mice and rats	Myco0771
Mycoplasma spp.	Otu04125	164	MT027176	90% *Mycoplasma ravipulmonis* (AF001173), grey lung disease agent in *Mus musculus*; 80% *M. phocidae* from California sea lions (DQ521594)	Myco4125
Eukaryotic family Sarcocystidae	Otu00056	8684	XXXXXX	97% similar to plastid small ribosomal unit of *Hyaloklossia lieberkuehni* (AF297120), a parasitic protazoa of European green frog; 96% *Noespora canim* (MK770339) & *Sarcocystis muris* (AF255924); 95% *Toxoplasma gondii* (TGU28056)	Sarcocystidae1
Eukaryotic family Sarcocystidae	Otu00191	3678	XXXXXX	92% *Neospora caninum* (MK770339) parasite; 90% *Toxoplasma gondii* (U87145) zoonotic pathogen	Sarcocystidae2
Eukaryotic family Sarcocystidae	Otu00254	1219	XXXXXX	98% *Sarcocystis muris* (AF255924) coccidian parasite first found in mice	Sarcocystidae3

773

774 Table 1 (continued). BLAST search results for OTUs suspected of belonging to pathogenic genera.
775

Infecting species identity	OTU Number	Nuimber of Reads	Genbank Accession Number	BLAST results (% identity)	Pathogen Code
Uncertin pathogenicity, reliably detectable					
Corynebacterium xerosis	Otu00050	1853	MT027177	100% *Corynebacterium xerosis* (MH141477), only opportunistic infections identified	Corynebacterium
Dietzia spp.	Otu00102	2626	MT027178	100% *Dietzia* spp. e.g., *D. aurantiaca* (MK25331); common contaminant; opportunistic in humans; thoguht to out-compete *Trypanosomes*	Dietzia
Helicobacter spp.	Otu00013	34894	MT027179	96% homology to *Helicobacter suncus* (AB006147) isolated from shrews with chronic gastritis; 95% identity to type speciment for *H. mustelae* (NR_029169) which causes gastritis in ferrets; but could be normal gut flora	Helico1
Helicobacter spp.	Otu00025	8702	MT027180	97% similar to *Helicobacter trogontum* (AY686609) and *H. sencus* (AB006147), both enterohepatic *Helicobacter* spp. associated with intestinal diseases	Helico2
Helicobacter spp.	Otu00087	2303	MT027181	99% identical to *Helicobacter aurati* (NR_025124.1), a pathogen of Syrian hamsters; 98% identical to *H. fennelliae* (GQ867176), a human pathogen	Helico3
Helicobacter spp.	Otu00128	1178	MT027182	99% *Helicobacter winghamensis* (AF363063), associated with gastroenteritis in humans ; however, minor sequences were 100% identical to *H. rodentium* (AY631957) which is only associated to gastritis in rodents when coinfecting with other *Helicobacter* strains	Helico4
Neisseria spp.	Otu00612	780	MT027183	97% uncultured *Neisseria* spp. assoicated with human prostatitis (HM080767) and cataracts (MG696979), but undistinguishable from environmental samples and healthy flora (e.g., JF139578)	Neisseria1
Pasteurellaceae	Otu00129	1430	MT027184	100% uncultured bacterium (MN095269) of mouse oral flora; 99% *Muribacter muris* (KP278064) of unknown pathogenicity, water fowl pathogen *Avibacterium gallinarum* (AF487729), and cattle respiratory disease agent *Mannheimia haemolytica* (CP017491)	Pasteurella1
Pasteurellaceae	Otu00203	521	MT027185	99% *Aggregatibacter aphrophilus* (LR134327) and *Haemophilus parainfluenzae* (CP035368) opportunistic pathogens but otherwise part of normal flora	Pasteurella2
Rickettsiella spp.	Otu00187	592	MT027186	99%-100% identity to several endosymbionts of insects, eg. uncultured *Diplorickettsia* spp. in sand flies (KX363696), *Rickettsiella* spp. in Ixodes ticks (KP994859); 99% identity to *Rickettsiella agriotidis* (HQ640943) pathogen of wireworms	Rickettsiella
Streptococcus spp.	Otu00115	1681	MT027187	99% *Streptococcus hyointestinalis* from intestines of swine (KR819489)	Streptococcus
Yersinia spp.	Otu00041	7420	MT027188	A heterogeneous OTU some major sequences 100% *Yersinia* spp. and *Serratia* spp., including pathogenic zoonotic bacteria (e.g., *Y. pestis* NR_025160) and non-pathogenic endosymbionts of plants (NR_157762) ; some major sequences 100% *Pantoea agglomerans* (MN515098) opportunists	Yersinia
Uncertin pathogenicity, not reliably detectable					
Fusobacterium spp.	Otu00791	108	MT027189	100% *Fusobacterium ulcerans* (CP028105) from tropical foot ulcers in humans; but also 100% identity with other fecal isolates of unknown pathogenicity in mammals (e.g., *F. varium* LR134390)	Fusobacterium
Neisseria spp.	Otu00454	148	MT027190	98% uncultured microbiota of bat mating organs (KY300287), 98% *Simonsiella muelleri* commensul from human saliva (AF328145); 97% *Kingella kingae* (MF073277) pathogen in humans	Neisseria2
Treponema spp.	Otu00235	348	MT027191	93% uncultured rumen *Treponema* spp. (AB537611)	Treponema
Williamsia spp.	Otu00614	274	MT027192	100% *Williamsia phyllospherae* (MG205541) and *Williamsia maris* (NR_024671), closely related to opportunistic pathogens in humans	Williamsia

776

References

Abbate, J.L., Ezenwa, V.O., Guégan, J.-F., Choisy, M., Nacher, M. & Roche, B. (2018) Disentangling complex parasite interactions: Protection against cerebral malaria by one helminth species is jeopardized by co-infection with another. *PLOS Neglected Tropical Diseases*, **12**, 1–13.

Aivelo, T. & Norberg, A. (2018) Parasite–microbiota interactions potentially affect intestinal communities in wild mammals. *Journal of Animal Ecology*, **87**, 438–447.

Andrews, C.H. & Glover, R.E. (1945) Grey Lung virus: an agent pathogenic for mice and other rodents. *British Journal of Experimental Pathology*, **26**, 379–387.

Bai, Y., Kosoy, M.Y., Lerdthusnee, K., Peruski, L.F. & Richardson, J.H. (2009) Prevalence and genetic heterogeneity of Bartonella strains cultured from rodents from 17 provinces in Thailand. *American Journal of Tropical Medicine and Hygiene*, **81**, 811–816.

Baker, D.G. (1998) Natural Pathogens of Laboratory Mice , Rats , and Rabbits and Their Effects on Research. *Clinical Microbiology Reviews*, **11**, 231–266.

Behnke, J.M. (2008) Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions .... or pure randomness? *Parasitology*, **135**, 751–766.

Beldomenico, P.M. & Begon, M. (2010) Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology & Evolution*, **25**, 21–27.

Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M. & Begon, M. (2008) Poor condition and infection: a vicious circle in natural populations. *Proceedings. Biological sciences*, **275**, 1753–1759.

Bennett, M., Crouch, A.J., Begon, M., Duffy, B., Feore, S., Gaskell, R.M., Kelly, D.F., McCracken, C.M., Vicary, L. & Baxby, D. (1997) Cowpox in British voles and mice. *Journal of Comparative Pathology*, **116**, 35–44.

Birtles, R.J., Hazel, S.M., Bennett, M., Bown, K., Raoult, D. & Begon, M. (2001) Longitudinal monitoring of the dynamics of infections due to Bartonella species in UK woodland rodents.

Epidemiology and infection, **126**, 323–329.

Bordes, F., Caron, A., Blasdell, K., de Garine-Wichatitsky, M. & Morand, S. (2017) Forecasting potential emergence of zoonotic diseases in South-East Asia: network analysis identifies key rodent hosts. *Journal of Applied Ecology*, **54**, 691–700.

Bordes, F., Herbreteau, V., Dupuy, S., Chaval, Y., Tran, A. & Morand, S. (2013) The diversity of microparasites of rodents: a comparative analysis that helps in identifying rodent-borne rich habitats in Southeast Asia. *Infection ecology & epidemiology*, **3**.

Breitschwerdt, E.B. (2014) Bartonellosis: One health perspectives for an emerging infectious disease. *ILAR Journal*, **55**, 46–58.

Breitschwerdt, E.B. & Kordick, D.L. (2000) Bartonella infection in animals: Carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbiology Reviews*, **13**, 428–438.

Brook, C.E., Bai, Y., Yu, E.O., Ranaivoson, H.C., Shin, H., Dobson, A.P., Metcalf, C.J.E., Kosoy, M.Y. & Dittmar, K. (2017) Elucidating transmission dynamics and host-parasite-vector relationships for rodent-borne Bartonella spp. in Madagascar. *Epidemics*, **20**, 56–66.

Brown, A.F. (1986) Evidence for density-dependent establishment and survival of Pomphorhynchus laevis (Müller, 1776) (Acanthocephala) in laboratory-infected Salmo gairdneri Richardson and its bearing on wild populations in Leuciscus cephalus (L.). *Journal of Fish Biology*, **28**, 659–669.

Budischak, S.A., Sakamoto, K., Megow, L.C., Cummings, K.R., Urban, J.F. & Ezenwa, V.O. (2015) Resource limitation alters the consequences of co-infection for both hosts and parasites. *International Journal for Parasitology*, **45**, 455–463.

Budischak, S.A., Wiria, A.E., Hamid, F., Wammes, L.J., Kaisar, M.M.M., van Lieshout, L., Sartono, E., Supali, T., Yazdanbakhsh, M. & Graham, A.L. (2018) Competing for blood: the ecology of parasite resource competition in human malaria–helminth co-infections. *Ecology Letters*, **21**, 536–545.

829 Buffet, J.-P., Pisanu, B., Brisse, S., Roussel, S., Félix, B., Halos, L., Chapuis, J.-L. & Vayssier-
830 Taussat, M. (2013) Deciphering Bartonella Diversity, Recombination, and Host Specificity in
831 a Rodent Community. *PLOS ONE*, **8**, e68956.
832 Burthe, S., Telfer, S., Lambin, X., Bennett, M., Carslake, D., Smith, A. & Begon, M. (2006)
833 Cowpox virus infection in natural field vole Microtus agrestis populations: delayed density
834 dependence and individual risk. *Journal of Animal Ecology*, **75**, 1416–1425.
835 Casadevall, A. & Pirofski, L.A. (2000) Host-pathogen interactions: basic concepts of microbial
836 commensalism, colonization, infection, and disease. *Infection and immunity*, **68**, 6511–6518.
837 Cattadori, I.M., Albert, R. & Boag, B. (2007) Variation in host susceptibility and infectiousness
838 generated by co-infection: the myxoma–Trichostrongylus retortaeformis case in wild rabbits.
839 *Journal of The Royal Society Interface*, **4**, 831–840.
840 Cattadori, I.M., Boag, B. & Hudson, P.J. (2008) Parasite co-infection and interaction as drivers of
841 host heterogeneity. *International Journal for Parasitology*, **38**, 371–380.
842 Cattadori, I.M., Haukisalmi, V., Henttonen, H. & Hudson, P.J. (2006) Transmission ecology and the
843 structure of parasite communities in small mammals BT - Micromammals and
844 Macroparasites: From Evolutionary Ecology to Management. (eds S. Morand, B.R. Krasnov &
845 R. Poulin), pp. 349–369. Springer Japan, Tokyo.
846 Charbonnel, N., Deter, J., Chaval, Y., Laakkonen, J., Henttonen, H., Voutilainen, L., Vapalahti, O.,
847 Vaheri, A., Morand, S. & Cosson, J.-F. (2008) Serological Evidence of Viruses Naturally
848 Associated with the Montane Water Vole (Arvicola scherman) in Eastern France. *Vector-*
849 *Borne and Zoonotic Diseases*, **8**, 763–768.
850 Charbonnel, N., Pagès, M., Sironen, T., Henttonen, H., Vapalahti, O., Mustonen, J. & Vaheri, A.
851 (2014) Immunogenetic factors affecting susceptibility of humans and rodents to hantaviruses
852 and the clinical course of hantaviral disease in humans. *Viruses*, **6**, 2214–2241.
853 Claesson, M.J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P. & O'Toole,
854 P.W. (2010) Comparison of two next-generation sequencing technologies for resolving highly

855 complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids*
856 *Research*, **38**, e200-213.
857 Clark, N.J., Wells, K., Dimitrov, D. & Clegg, S.M. (2016) Co-infections and environmental
858 conditions drive the distributions of blood parasites in wild birds. *Journal of Animal Ecology*,
859 **85**, 1461–1470.
860 Cleaveland, S., Laurenson, M.K. & Taylor, L.H. (2001) Diseases of humans and their domestic
861 mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical*
862 *transactions of the Royal Society of London. Series B, Biological sciences*, **356**, 991–999.
863 Cohen, C., Einav, M. & Hawlena, H. (2015) Path analyses of cross-sectional and longitudinal data
864 suggest that variability in natural communities of blood-associated parasites is derived from
865 host characteristics and not interspecific interactions. *Parasites & vectors*, **8**, 429.
866 Cohen, C., Shemesh, M., Garrido, M., Messika, I., Einav, M., Khokhlova, I., Tasker, S. & Hawlena,
867 H. (2018) Haemoplasmas in wild rodents: Routes of transmission and infection dynamics.
868 *Molecular Ecology*, **27**, 3714–3726.
869 Cougoul, A., Bailly, X., Vourc'h, G. & Gasqui, P. (2019) Rarity of microbial species: In search of
870 reliable associations. *PLOS ONE*, **14**, e0200458.
871 Cox, F.E.G. (2001) Concomitant infections , parasites and immune responses. *Parasitology*, **122**,
872 S23–S38.
873 Dallas, T.A., Laine, A.-L. & Ovaskainen, O. (2019) Detecting parasite associations within multi-
874 species host and parasite communities. *Proceedings of the Royal Society B: Biological*
875 *Sciences*, **286**, 20191109.
876 Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000) Emerging Infectious Diseases of Wildlife -
877 Threats to Biodiversity and Human Health. *Science*, **287**, 443–449.
878 Davies, T.J. & Pedersen, A.B. (2008) Phylogeny and geography predict pathogen community
879 similarity in wild primates and humans. *Proceedings. Biological sciences / The Royal Society*,
880 **275**, 1695–701.

881 Davis, S., Calvet, E. & Leirs, H. (2005) Fluctuating Rodent Populations and Risk to Humans from
882 Rodent-Borne Zoonoses. *Vector-Borne and Zoonotic Diseases*, **5**, 305–314.
883 Defosse, D.L., Johnson, R.C., Paster, B.J., Dewhirst, F.E. & Fraser, G.J. (1995) Brevinema
884 andersonii gen. nov., sp. nov., an infectious spirochete isolated from the short-tailed shrew
885 (Blarina brevicauda) and the white-footed mouse (Peromyscus leucopus). *International journal*
886 *of systematic bacteriology*, **45**, 78–84.
887 Deter, J., Chaval, Y., Galan, M., Gauffre, B., Morand, S., Henttonen, H., Laakkonen, J.,
888 Voutilainen, L., Charbonnel, N. & Cosson, J.-F. (2007) Kinship, dispersal and hantavirus
889 transmission in bank and common voles. *Archives of Virology*, **153**, 435.
890 Diacovich, L. & Gorvel, J.-P. (2010) Bacterial manipulation of innate immunity to promote
891 infection. *Nature Reviews Microbiology*, **8**, 117–128.
892 Diagne, C., Galan, M., Tamisier, L., d'Ambrosio, J., Dalecky, A., Bâ, K., Kane, M., Niang, Y.,
893 Diallo, M., Sow, A., Gauthier, P., Tatard, C., Loiseau, A., Piry, S., Sembène, M., Cosson, J.-F.,
894 Charbonnel, N. & Brouat, C. (2017) Ecological and sanitary impacts of bacterial communities
895 associated to biological invasions in African commensal rodent communities. *Scientific*
896 *reports*, **7**, 14995.
897 Eidelman, A., Cohen, C., Navarro-Castilla, Á., Filler, S., Gutiérrez, R., Bar-Shira, E., Shahar, N.,
898 Garrido, M., Halle, S., Romach, Y., Barja, I., Tasker, S., Harrus, S., Friedman, A. & Hawlena,
899 H. (2019) The dynamics between limited-term and lifelong coinfecting bacterial parasites in
900 wild rodent hosts. *The Journal of Experimental Biology*, **222**, jeb203562.
901 Essbauer, S., Pfeffer, M. & Meyer, H. (2010) Zoonotic poxviruses. *Veterinary Microbiology*, **140**,
902 229–236.
903 Ezenwa, V.O., Etienne, R.S., Luikart, G., Beja-Pereira, A. & Jolles, A.E. (2010) Hidden
904 Consequences of Living in a Wormy World: Nematode-Induced Immune Suppression
905 Facilitates Tuberculosis Invasion in African Buffalo. *The American Naturalist*, **176**, 613–624.
906 Ezenwa, V.O. & Jolles, A.E. (2015) Opposite effects of anthelmintic treatment on microbial

907 infection at individual versus population scales. *Science*, **347**, 175–177.

908 Fenton, A. (2008) Worms and germs: the population dynamic consequences of microparasite-
909 macroparasite co-infection. *Parasitology*, **135**, 1545–1560.

910 Fenton, A., Knowles, S.C.L., Petchey, O.L. & Pedersen, A.B. (2014) The reliability of
911 observational approaches for detecting interspecific parasite interactions: Comparison with
912 experimental results. *International Journal for Parasitology*, **44**, 437–445.

913 Fenton, A., Viney, M.E. & Lello, J. (2010) Detecting interspecific macroparasite interactions from
914 ecological data: Patterns and process. *Ecology Letters*, **13**, 606–615.

915 Fettweis, J.M., Serrano, M.G., Huang, B., Brooks, J.P., Glascock, A.L., Sheth, N.U., Consortium,
916 V.M., Strauss 3rd, J.F., Jefferson, K.K. & Buck, G.A. (2014) An emerging mycoplasma
917 associated with trichomoniasis, vaginal infection and disease. *PloS one*, **9**, e110943–e110943.

918 Forbes, K.M., Voutilainen, L., Jääskeläinen, A., Sironen, T., Kinnunen, P.M., Stuart, P., Vapalahti,
919 O., Henttonen, H. & Huitu, O. (2014) Serological survey of rodent-borne viruses in Finnish
920 field voles. *Vector borne and zoonotic diseases (Larchmont, N.Y.)*, **14**, 278–283.

921 Fountain-Jones, N.M., Packer, C., Jacquot, M., Blanchet, F.G., Terio, K. & Craft, M.E. (2019)
922 Endemic infection can shape exposure to novel pathogens: Pathogen co-occurrence networks
923 in the Serengeti lions. *Ecology Letters*, **22**, 904–913.

924 Galan, M., Razzauti, M., Bard, E., Bernard, M., Brouat, C., Charbonnel, N., Dehne-Garcia, A.,
925 Loiseau, A., Tatard, C., Tamisier, L., Vayssier-Taussat, M., Vignes, H. & Cosson, J.-F. (2016)
926 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife.
927 *mSystems*, **1**, e00032-16.

928 Garchitorena, A., Sokolow, S.H., Roche, B., Ngonghala, C.N., Jocque, M., Lund, A., Barry, M.,
929 Mordecai, E.A., Daily, G.C., Jones, J.H., Bonds, M.H., Leo, G.A. De & Gue, J.F. (2017)
930 Disease ecology , health and the environment : a framework to account for ecological and
931 socio-economic drivers in the control of neglected tropical diseases. *Philosophical*
932 *Transactions of the Royal Society B*, **372**, e20160128.

933 Gaulke, C.A., Martins, M.L., Watral, V.G., Humphreys, I.R., Spagnoli, S.T., Kent, M.L. &
934 Sharpton, T.J. (2019) A longitudinal assessment of host-microbe-parasite interactions resolves
935 the zebrafish gut microbiome's link to Pseudocapillaria tomentosa infection and pathology.
936 *Microbiome*, **7**, 10.
937 Gorsich, E.E., Ezenwa, V.O. & Jolles, A.E. (2014) Nematode-coccidia parasite co-infections in
938 African buffalo: Epidemiology and associations with host condition and pregnancy.
939 *International Journal for Parasitology: Parasites and Wildlife*, **3**, 124–134.
940 Gotteland, C., Chaval, Y., Villena, I., Galan, M., Geers, R., Aubert, D., Poulle, M.-L., Charbonnel,
941 N. & Gilot-Fromont, E. (2014) Species or local environment, what determines the infection of
942 rodents by *Toxoplasma gondii*? *Parasitology*, **141**, 259–268.
943 Graham, J.E. & Schoeb, T.R. (2011) Mycoplasma pulmonis in Rats. *Journal of Exotic Pet*
944 *Medicine*, **20**, 270–276.
945 Griffiths, E.C., Pedersen, A.B., Fenton, A., Petchey, O.L. & Sheffield, S. (2011) The nature and
946 consequences of coinfection in humans. *The Journal of infection*, **63**, 200–6.
947 Guivier, E., Galan, M., Henttonen, H., Cosson, J.-F. & Charbonnel, N. (2014) Landscape features
948 and helminth co-infection shape bank vole immunoheterogeneity, with consequences for
949 Puumala virus epidemiology. *Heredity*, **112**, 274–281.
950 Guy, L., Nystedt, B., Toft, C., Zaremba-Niedzwiedzka, K., Berglund, E.C., Granberg, F., Näslund,
951 K., Eriksson, A.S. & Andersson, S.G.E. (2013) A Gene Transfer Agent and a Dynamic
952 Repertoire of Secretion Systems Hold the Keys to the Explosive Radiation of the Emerging
953 Pathogen Bartonella. *PLoS Genetics*, **9**, e1003393.
954 Guy, C., Thiagavel, J., Mideo, N. & Ratcliffe, J.M. (2019) Phylogeny matters: revisiting 'a
955 comparison of bats and rodents as reservoirs of zoonotic viruses.' *Royal Society Open Science*,
956 **6**, 181182.
957 Han, B.A., Schmidt, J.P., Bowden, S.E. & Drake, J.M. (2015) Rodent reservoirs of future zoonotic
958 diseases. *Proceedings of the National Academy of Sciences*, **112**, 7039–7044.

959 Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. & Samuel, M.D.
960 (2002) Climate warming and disease risks for terrestrial and marine biota. *Science*, **296**, 2158–
961 2162.
962 Harwick, H.J., Kalmanson, G.M. & Guze, L.B. (1972) Human diseases associated with
963 Mycoplasmas. *California Medicine*, **116**, 1–7.
964 Iralu, J., Bai, Y., Crook, L., Tempest, B., Simpson, G., McKenzie, T. & Koster, F. (2006) Rodent-
965 associated Bartonella febrile illness, southwestern United States. *Emerging Infectious*
966 *Diseases*, **12**, 1081–1086.
967 Johnston, J.B. & McFadden, G. (2003) Poxvirus Immunomodulatory Strategies: Current
968 Perspectives. *Journal of Virology*, **77**, 6093 LP – 6100.
969 Jolles, A.E., Ezenwa, V.O., Etienne, R.S., Turner, W.C. & Olff, H. (2008) Interactions between
970 macroparasites and microparasites drive infection patterns in free-ranging african buffalo.
971 *Ecology*, **89**, 2239–2250.
972 Jones, K.E., Patel, N.G., Levy, M. a, Storeygard, A., Balk, D., Gittleman, J.L. & Daszak, P. (2008)
973 Global trends in emerging infectious diseases. *Nature*, **451**, 990–3.
974 Kallio-Kokko, H., Laakkonen, J., Rizzoli, A., Tagliapietra, V., Cattadori, I., Perkins, S.E., Hudson,
975 P.J., Cristofolini, A., Versini, W., Vapalahti, O., Vaheri, A. & Henttonen, H. (2006)
976 Hantavirus and arenavirus antibody prevalence in rodents and humans in Trentino, Northern
977 Italy. *Epidemiology and Infection*, **134**, 830.
978 Kedem, H., Cohen, C., Messika, I., Einav, M., Pilosof, S. & Hawlena, H. (2014) Multiple effects of
979 host-species diversity on coexisting host-specific and host-opportunistic microbes. *Ecology*,
980 **95**, 1173–1183.
981 Knowles, S.C.L., Fenton, A., Petchey, O.L., Jones, T.R., Barber, R. & Pedersen, A.B. (2013)
982 Stability of within-host-parasite communities in a wild mammal system. *Proceedings.*
983 *Biological sciences*, **280**, 20130598.
984 Koskela, K.A., Kalin-Mänttäri, L., Hemmilä, H., Smura, T., Kinnunen, P.M., Niemimaa, J.,

985 Henttonen, H. & Nikkari, S. (2016) Metagenomic Evaluation of Bacteria from Voles. *Vector-*
986 *Borne and Zoonotic Diseases*, **17**, 123–133.
987 Kosoy, M., Mandel, E., Green, D., Marston, E., Jones, D. & Childs, J. (2004) Prospective Studies of
988 Bartonella of Rodents. Part II. Diverse Infections in a Single Rodent Community. *Vector-*
989 *Borne and Zoonotic Diseases*, **4**, 296–305.
990 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013) Development of
991 a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data
992 on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, **79**,
993 5112–5120.
994 Krasnov, B.R., Mouillot, D., Shenbrot, G.I., Khokhlova, I.S. & Poulin, R. (2010) Deconstructing
995 spatial patterns in species composition of ectoparasite communities: the relative contribution of
996 host composition, environmental variables and geography. *Global Ecology and Biogeography*,
997 **19**, 515–526.
998 Kreisinger, J., Bastien, G., Hauffe, H.C., Marchesi, J. & Perkins, S.E. (2015) Interactions between
999 multiple helminths and the gut microbiota in wild rodents. *Philosophical transactions of the*
1000 *Royal Society of London. Series B, Biological sciences*, **370**.
1001 Lello, J., Boag, B., Fenton, A., Stevenson, I.R. & Hudson, P.J. (2004) Competition and mutualism
1002 among the gut helminths of a mammalian host. *Nature*, **428**, 840–844.
1003 Lello, J., McClure, S.J., Tyrrell, K. & Viney, M.E. (2018) Predicting the effects of parasite co-
1004 infection across species boundaries. *Proceedings. Biological sciences*, **285**, 20172610.
1005 Luis, A.D., Hayman, D.T.S., O'Shea, T.J., Cryan, P.M., Gilbert, A.T., Pulliam, J.R.C., Mills, J.N.,
1006 Timonin, M.E., Willis, C.K.R., Cunningham, A.A., Fooks, A.R., Rupprecht, C.E., Wood,
1007 J.L.N. & Webb, C.T. (2013) A comparison of bats and rodents as reservoirs of zoonotic
1008 viruses: are bats special? *Proceedings. Biological sciences*, **280**, 20122753.
1009 Matar, G.M., Koehler, J.E., Malcolm, G., Lambert-Fair, M.A., Tappero, J., Hunter, S.B. &
1010 Swaminathan, B. (1999) Identification of Bartonella species directly in clinical specimens by

1011 PCR-restriction fragment length polymorphism analysis of a 16S rRNA gene fragment.
1012 *Journal of Clinical Microbiology*, **37**, 4045–4047.
1013 Messick, J.B. (2004) Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into
1014 pathogenic potential. *Veterinary clinical pathology / American Society for Veterinary Clinical*
1015 *Pathology*, **33**, 2–13.
1016 Meyer, B.J. & Schmaljohn, C.S. (2000) Persistent hantavirus infections: characteristics and
1017 mechanisms. *Trends in Microbiology*, **8**, 61–67.
1018 Morand, S. (2015) (macro-) Evolutionary ecology of parasite diversity: From determinants of
1019 parasite species richness to host diversification. *International journal for parasitology.*
1020 *Parasites and wildlife*, **4**, 80–87.
1021 Morand, S. & Bordes, F. (2015) Parasite diversity of disease-bearing rodents of Southeast Asia:
1022 habitat determinants and effects on sexual size dimorphism and life-traits. *Frontiers in Ecology*
1023 *and Evolution*, **3**, 110.
1024 Mouillot, D., Šimková, A., Morand, S. & Poulin, R. (2005) Parasite species coexistence and
1025 limiting similarity: a multiscale look at phylogenetic, functional and reproductive distances.
1026 *Oecologia*, **146**, 269–278.
1027 Moutailler, S., Moro, C.V., Vaumourin, E. & Michelet, L. (2016) Co-infection of Ticks : The Rule
1028 Rather Than the Exception. *PLoS Neglected Tropical Diseases*, **10**, e0004539.
1029 Murall, C.L., Abbate, J.L., Puelma Touzel, M., Allen-Vercoe, E., Alizon, S., Froissart, R. &
1030 McCann, K. (2017) *Invasions of Host-Associated Microbiome Networks*, 1st ed. Elsevier Ltd.
1031 Neimark, H., Johansson, K.E., Rikihisa, Y. & Tully, J.G. (2001) Proposal to transfer some members
1032 of the genera Haemobartonella and Eperythrozoon to the genus Mycoplasma with descriptions
1033 of "*Candidatus* Mycoplasma haemofelis", "*Candidatus* Mycoplasma haemomuris",
1034 "*Candidatus* Mycoplasma haemosuis" a. *International Journal of Systematic and Evolutionary*
1035 *Microbiology*, **51**, 891–899.
1036 Neimark, H., Mitchelmore, D. & Leach, R.H. (1998) An approach to characterizing uncultivated

1037 prokaryotes: the Grey Lung agent and proposal of a *Candidatus* taxon for the organism,
1038 "*Candidatus* Mycoplasma ravipulmonis". *International journal of systematic bacteriology*, **48**
1039 **Pt 2**, 389–94.
1040 Neimark, H., Peters, W., Robinson, B.L. & Stewart, L.B. (2005) Phylogenetic analysis and
1041 description of Eperythrozoon coccoides, proposal to transfer to the genus Mycoplasma as
1042 Mycoplasma coccoides comb. nov. and request for an opinion. *International Journal of*
1043 *Systematic and Evolutionary Microbiology*, **55**, 1385–1391.
1044 Nunn, C.L., Altizer, S., Jones, K.E. & Sechrest, W. (2003) Comparative Tests of Parasite Species
1045 Richness in Primates. *The American Naturalist*, **162**, 597–614.
1046 Pawelczyk, A., Bajer, A., Behnke, J.M., Gilbert, F.S. & Sinski, E. (2004) Factors affecting the
1047 component community structure of haemoparasites in common voles (Microtus arvalis) from
1048 the Mazury Lake District region of Poland. *Parasitology Research*, **92**, 270–284.
1049 Pedersen, A.B., Altizer, S., Poss, M., Cunningham, A.A. & Nunn, C.L. (2005) Patterns of host
1050 specificity and transmission among parasites of wild primates. *International Journal for*
1051 *Parasitology*, **35**, 647–657.
1052 Pedersen, A.B. & Fenton, A. (2007) Emphasizing the ecology in parasite community ecology.
1053 *Trends in Ecology and Evolution*, **22**, 133–139.
1054 Petney, T.N. & Andrews, R.H. (1998) Multiparasite communities in animals and humans:
1055 frequency, structure and pathogenic significance. *International Journal for Parasitology*, **28**,
1056 377–393.
1057 Piasecki, T., Chrzastek, K. & Kasprzykowska, U. (2017) Mycoplasma pulmonis of Rodents as a
1058 Possible Human Pathogen. *Vector-Borne and Zoonotic Diseases*, **17**, 475–477.
1059 Pilosof, S., Morand, S., Krasnov, B.R. & Nunn, C.L. (2015) Potential Parasite Transmission in
1060 Multi-Host Networks Based on Parasite Sharing. *PLOS ONE*, **10**, 1–19.
1061 Pitcher, D.G. & Nicholas, R.A.J. (2005) Mycoplasma host specificity: Fact or fiction? *Veterinary*
1062 *Journal*, **170**, 300–306.

1063 Poulin, R. (1995) Phylogeny, ecology, and the richness of parasite communities in vertebrates.
1064 *Ecological Monographs*, **65**, 283–302.
1065 Poulin, R., Blanar, C.A., Thieltges, D.W. & Marcogliese, D.J. (2012) Scaling up from
1066 epidemiology to biogeography: local infection patterns predict geographical distribution in fish
1067 parasites. *Journal of Biogeography*, **39**, 1157–1166.
1068 Poulin, R. & Morand, S. (2000) The Diversity of Parasites. *The Quarterly Review of Biology*, **75**,
1069 277–293.
1070 R Core Team. (2015) R: A language and environment for statistical computing.
1071 Razzauti, M., Galan, M., Bernard, M., Maman, S., Klopp, C., Charbonnel, N., Vayssier-Taussat,
1072 M., Eloit, M. & Cosson, J.-F. (2015) A Comparison between Transcriptome Sequencing and
1073 16S Metagenomics for Detection of Bacterial Pathogens in Wildlife. *PLOS Neglected Tropical*
1074 *Diseases*, **9**, 1–21.
1075 Salisbury, A.M., Begon, M., Dove, W., Niklasson, B. & Stewart, J.P. (2014) Ljungan virus is
1076 endemic in rodents in the UK. *Archives of Virology*, **159**, 547–551.
1077 Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P.,
1078 Parkhill, J., Loman, N.J. & Walker, A.W. (2014) Reagent and laboratory contamination can
1079 critically impact sequence-based microbiome analyses. *BMC Biology*, **12**, 87.
1080 Salvador, A.R., Guivier, E., Xuéreb, A., Chaval, Y., Cadet, P., Poulle, M.-L., Sironen, T.,
1081 Voutilainen, L., Henttonen, H., Cosson, J.-F. & Charbonnel, N. (2011) Concomitant influence
1082 of helminth infection and landscape on the distribution of Puumala hantavirus in its reservoir,
1083 Myodes glareolus. *BMC Microbiology*, **11**, 30.
1084 Sauvage, F., Penalba, C., Vuillaume, P., Boue, F., Coudrier, D., Pontier, D. & Artois, M. (2002)
1085 Puumala hantavirus infection in humans and in the reservoir host, Ardennes region, France.
1086 *Emerging infectious diseases*, **8**, 1509–1511.
1087 Singer, M. (2010) Pathogen-pathogen interaction: A syndemic model of complex biosocial
1088 processes in disease. *Virulence*, **1**, 10–18.

1089 Smith, K.F., Sax, D.O.V.F. & Lafferty, K.D. (2006) Evidence for the Role of Infectious Disease in
1090 Species Extinction and Endangerment. *Conservation Biology*, **20**, 1349–1357.
1091 Streicker, D.G., Fenton, A. & Pedersen, A.B. (2013) Differential sources of host species
1092 heterogeneity influence the transmission and control of multihost parasites. *Ecology Letters*,
1093 **16**, 975–984.
1094 Streicker, D.G., Turmelle, A.S., Vonhof, M.J., Kuzmin, I. V, McCracken, G.F. & Rupprecht, C.E.
1095 (2010) Host phylogeny constrains cross-species emergence and establishment of rabies virus in
1096 bats. *Science (New York, N.Y.)*, **329**, 676–9.
1097 Stutz, W.E., Blaustein, A.R., Briggs, C.J., Hoverman, J.T., Rohr, J.R. & Johnson, P.T.J. (2018)
1098 Using multi-response models to investigate pathogen coinfections across scales: Insights from
1099 emerging diseases of amphibians. *Methods in Ecology and Evolution*, **9**, 1109–1120.
1100 Swanson, S.J., Neitzel, D., Reed, K.D. & Belongia, E.A. (2006) Coinfections acquired from ixodes
1101 ticks. *Clinical microbiology reviews*, **19**, 708–727.
1102 Sykes, J.E., Terry, J.C., Lindsay, L.L. & Owens, S.D. (2008) Prevalences of various hemoplasma
1103 species among cats in the United States with possible hemoplasmosis. *Journal of the American*
1104 *Veterinary Medical Association*, **232**, 372–379.
1105 Tadin, A., Turk, N., Korva, M., Margaletić, J., Beck, R., Vucelja, M., Habuš, J., Svoboda, P.,
1106 Zupanc, T.A., Henttonen, H. & Markotić, A. (2012) Multiple co-infections of rodents with
1107 hantaviruses, Leptospira, and Babesia in Croatia. *Vector borne and zoonotic diseases*
1108 *(Larchmont, N.Y.)*, **12**, 388–392.
1109 Tagawa, M., Takeuchi, T., Fujisawa, T., Konno, Y., Yamamoto, S., Matsumoto, K., Yokoyama, N.
1110 & Inokuma, H. (2012) A Clinical Case of Severe Anemia in a Sheep Coinfected with
1111 Mycoplasma ovis and `*Candidatus* Mycoplasma haemovis' in Hokkaido, Japan. *Journal of*
1112 *Veterinary Medical Science*, **74**, 99–102.
1113 Taylor, L.H., Latham, S.M. & Woolhouse, M.E.J. (2001) Risk factors for human disease
1114 emergence. *Philosophical Transactions of the Royal Society London B*, **356**, 983–989.

1115 Tegshduuren, E., Yoshimatsu, K., Taruishi, M., Endo, R., Shimizu, K., Koma, T., Yasuda, S.P.,
1116 Kariwa, H., Arikawa, J. & Ishihara, C. (2010) Different cross-reactivity of human and rodent
1117 sera to Tula virus and Puumala virus. *Comparative Immunology, Microbiology and Infectious*
1118 *Diseases*, **33**, e67–e73.
1119 Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S. & Begon, M. (2010)
1120 Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population.
1121 *Science*, **330**, 243–246.
1122 Thumbi, S.M., De. C. Bronsvoort, B.M., Poole, E.J., Kiara, H., Toye, P., Ndila, M., Conradie, I.,
1123 Jennings, A., Handel, I.G., Coetzer, J.A.W., Hanotte, O. & Woolhouse, M.E.J. (2013) Parasite
1124 co-infections show synergistic and antagonistic interactions on growth performance of East
1125 African zebu cattle under one year. *Parasitology*, **140**, 1789–1798.
1126 Vaheri, A., Strandin, T., Hepojoki, J., Sironen, T., Henttonen, H., Mäkelä, S. & Mustonen, J. (2013)
1127 Uncovering the mysteries of hantavirus infections. *Nature Reviews Microbiology*, **11**, 539–
1128 550.
1129 Vaumourin, E., Vourc'h, G., Telfer, S., Lambin, X., Salih, D., Seitzer, U., Morand, S., Charbonnel,
1130 N., Vayssier-Taussat, M. & Gasqui, P. (2014) To be or not to be associated: power study of
1131 four statistical modeling approaches to identify parasite associations in cross-sectional studies.
1132 *Frontiers in Cellular and Infection Microbiology*, **4**, 1–11.
1133 Vayssier-Taussat, M., Moutailler, S., F??m??nia, F., Raymond, P., Croce, O., La Scola, B.,
1134 Fournier, P.E. & Raoult, D. (2016) Identification of novel zoonotic activity of bartonella spp.,
1135 France. *Emerging Infectious Diseases*, **22**, 457–462.
1136 Vayssier-Taussat, M., Le Rhun, D., Buffet, J.-P., Maaoui, N., Galan, M., Guivier, E., Charbonnel,
1137 N. & Cosson, J.-F. (2012) *Candidatus* Neoehrlichia mikurensis in bank voles, France.
1138 *Emerging infectious diseases*, **18**, 2063–2065.
1139 Villette, P., Afonso, E., Couval, G., Levret, A., Galan, M., Tatard, C., Cosson, J.F. & Giradoux, P.
1140 (2017) Consequences of organ choice in describing bacterial pathogen assemblages in a rodent

1141 population. *Epidemiology and Infection*, **145**, 3070–3075.

1142 Volokhov, D. V, Hwang, J., Chizhikov, V.E., Danaceau, H. & Gottdenker, N.L. (2017) Prevalence,
1143 Genotype Richness, and Coinfection Patterns of Hemotropic Mycoplasmas in Raccoons
1144 (Procyon lotor) on Environmentally Protected and Urbanized Barrier Islands. *Applied and*
1145 *environmental microbiology*, **83**, e00211-17.

1146 Weiss, S., Van Treuren, W., Lozupone, C., Faust, K., Friedman, J., Deng, Y., Xia, L.C., Xu, Z.Z.,
1147 Ursell, L., Alm, E.J., Birmingham, A., Cram, J.A., Fuhrman, J.A., Raes, J., Sun, F., Zhou, J. &
1148 Knight, R. (2016) Correlation detection strategies in microbial data sets vary widely in
1149 sensitivity and precision. *The ISME Journal*, **10**, 1669–1681.

1150 Withenshaw, S.M., Devevey, G., Pedersen, A.B. & Fenton, A. (2016) Multihost Bartonella
1151 parasites display covert host specificity even when transmitted by generalist vectors. *The*
1152 *Journal of animal ecology*, **85**, 1442–1452.

1153 Woolhouse, M.E.J., Taylor, L.H. & Haydon, D.T. (2001) Population Biology of Multihost
1154 Pathogens. *Science*, **292**, 1109–1112.

1155 Yanagihara, R., Amyx, H.L. & Gajdusek, D.C. (1985) Experimental infection with Puumala virus,
1156 the etiologic agent of nephropathia epidemica, in bank voles (Clethrionomys glareolus).
1157 *Journal of virology*, **55**, 34–38.

1158 Zemb, O., Achard, C.S., Hamelin, J., De Almeida, M.-L., Gabinaud, B., Cauquil, L., Verschuren,
1159 L.M.G. & Godon, J.-J. (2020) Absolute quantitation of microbes using 16S rRNA gene
1160 metabarcoding: A rapid normalization of relative abundances by quantitative PCR targeting a
1161 16S rRNA gene spike-in standard. *MicrobiologyOpen*, **n/a**, e977.

1162

1163