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 occurrence 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

jessie abbate 12/3/20 20:05
Deleted: incidence

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. Factors extrinsic to the hosts 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). Factors extrinsic to the pathogens 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

jessie abbate 2/1/21 00:02
Deleted: Extrinsic factors

jessie abbate 2/1/21 00:03
Deleted:

jessie abbate 2/6/21 22:57
Deleted: (e.g., (Harvell et al. 2002; Burthe et al. 2006; Poulin et al. 2012))

jessie abbate 2/1/21 00:05
Deleted: Intrinsic factors

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), and prior infections can have lasting effects on future susceptibility via e.g., changes to host condition and behavior or through immune-mediated processes (Singer 2010; Quiñones-Parra *et al.* 2016; Kumar *et al.* 2018). Interactions among co-circulating parasites may have important consequences for disease severity, transmission and community-level responses to perturbations (Jolles *et al.* 2008; Telfer *et al.* 2010). Consequences of interaction may be life-long, as exposure to pathogens circulating among juveniles have been found to be strongly associated with those experienced by adults (e.g., Fountain-Jones *et al*. 2019). Such interactions can also play a role in the consequences of pathogen emergence (e.g., emerging bacterial infection increasing susceptibility to an endemic virus (Beechler *et al*. 2015)). 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. Strong ecological interactions have been shown in wild rodent populations among some of these zoonotic agents (Telfer *et al.* 2010), as well as between non-zoonotic agents and zoonotic agents (e.g., helminthes and bacteria (Carvalho-Pereira *et al.* 2019); helminthes and viruses (Guivier *et al.* 2014; Sweeny *et al.* 2020);

jessie abbate 1/30/21 07:09
Deleted: infecting

jessie abbate 1/28/21 15:10
Deleted: Among the most important zoonotic pathogens, we find *Echinococcus* spp., *Schistosoma* spp., *Toxoplasma* spp., *Trypanosoma* spp., *Yersinia pestis*, *Leptospira* spp., *Bartonella* spp., hantaviruses (e.g. Puumala virus) and arenaviruses (e.g. *Lassa virus*) in particular (https://www.gideononline.com/).

jessie abbate 1/28/21 15:11
Deleted: pathogenic taxa

jessie abbate 1/28/21 15:41
Deleted: micro- and macroparasites

helminthes and protozoa (Knowles *et al.* 2013)). 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 antiviral 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 of host splenic tissue). 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

jessie abbate 1/28/21 15:42
Deleted: (Salvador *et al.* 2011; Ezenwa & Jolles 2015)

jessie abbate 1/28/21 16:00
Deleted: 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).

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') (Supplemental Materials 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. sylvaticus* hosts; Figure S2B). Five *Microtus subterraneus* (European pine vole) and one each of

jessie abbate 2/21/21 12:19
Deleted: Supporting Informa

jessie abbate 2/21/21 12:19
Deleted: tion

jessie abbate 2/6/21 22:59
Deleted: *ly*

three additional host species (one cricetid one echimyid and one murid) 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 Supplemental Materials Appendix 1.

2.2. Detecting virus exposure and bacterial infection

Among the 713 rodents sampled for this study, indirect fluorescent antibody tests (IFATs; see for details (Kallio-Kokko *et al.* 2006)) were successfully performed on 677 animals to detect immunoglobulin G (IgG) specific to or cross-reacting with cowpox virus (CPXV, *Orthopoxvirus*), Puumala or Dobrava-Belgrade virus (respectively PUUV and DOBV, *Orthohantavirus*, collectively referred to henceforth as "hantavirus"), lymphocytic choriomeningitis virus (LCMV, *Mammarenavirus*), and Tick-borne encephalitis virus (TBEV, *Flavivirus*). We refer to these antiviral antibody tests as indicating a history of past exposure, but antibodies against hantavirus and LCMV also likely indicate continued chronic infection. In contrast, current or very recent exposure to bacterial infection was tested via 16S rRNA gene amplicon sequencing of splenic tissue, giving no indication of past exposure history. The spleen was chosen because this organ is known to filter microbial cells in mammals, allowing the detection of a wide array of pathogenic and zoonotic agents. Funding was available to test for bacteria in just half of the animals, chosen haphazardly to equally represent all host species, study sites and years, resulting in successful analysis for 332 rodents (see Figure 1 for a breakdown of number of individuals sampled per focal host species). For each individual animal, the DNA from splenic tissue was extracted using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer recommendations. Each DNA extraction was analyzed twice independently. We followed the method described in Galan *et al.* (2016) to perform PCR amplification, indexing, pooling, multiplexing, de-multiplexing, taxonomic identification using the SILVA SSU Ref NR 119 database as a reference (http://www.arb-silva.de/projects/ssu-ref-nr/). Briefly, DNA samples were amplified by PCR using universal primers targeting the hyper variable region V4 of the 16S rRNA gene (251 bp) and sequencing via Illumina

Jessie Abbate 2/21/21 13:58
Deleted: and two

Jessie Abbate 2/21/21 13:59
Deleted: s

Jessie Abbate 2/21/21 13:04
Deleted: Supporting

Jessie Abbate 2/21/21 13:04
Deleted: S

jessie abbate 2/5/21 13:37
Deleted: antibodies

jessie abbate 1/27/21 00:42
Deleted:

MiSeq. The V4 region has been proven to have reasonable taxonomic resolution at the genus level (Claesson *et al.* 2010). A multiplexing strategy enabled the identification of bacterial genera in each individual sample (Kozich *et al.* 2013). Data filtering was performed as described in Galan *et al.* (2016) to determine presence/absence of bacterial infections (summarized in Figure S3). Briefly, we discarded all bacterial OTUs containing fewer than 50 reads in the entire dataset and animals for which one or both individual PCR samples produced fewer than 500 reads. A bacterial OTU was considered present in an animal if the two independent PCR samples were both above a threshold number of reads, defined as the greater of either 0.012% of the total number of reads in the run for that OTU (i.e., filtering using the rate of indexing leak) or the maximum number of reads for that OTU in any negative control sample (i.e., filtering using the presence of reads in the negative controls due to contaminations) (Galan *et al.* 2016). We removed chimera using the *Uchime* program implemented in *mothur*, and manually checked OTUs representing suspected chimera not identified by the program. For each OTU suspected as pathogenic, Basic Local Alignment Search Tool (BLAST) searches of the most common sequences were conducted to infer species identity where possible. OTUs with at least 500 reads across all animals in the dataset were considered reliably detectable, allowing us to assign absent status to these OTUs in animals failing to meet the criteria for OTU presence. Only OTUs for which there were at least 500 reads across all animals in the dataset (for which present and absent statuses could be assigned), and where reasonable certainty of pathogenicity could be established from the literature, were considered in analyses of the pathogen community.

jessie abbate 2/6/21 23:01
Formatted: Font:Italic

2.3. Statistical Methods

All statistical analyses were implemented in R version 3.2.2 (R Core Team 2015). Throughout our analyses, we refer to simultaneous bacterial infections as "co-infection", while analyses involving simultaneous presence of antiviral antibodies and bacterial infection are referred to as evaluating "co-exposure". While we can only be sure that antiviral antibodies represent past

exposure, we cannot rule out simultaneous viral and bacterial "co-infection", particularly for viruses known to cause chronic infections. Likewise, detection of current (or very recent) bacterial infection, particularly for taxa known to cause chronic infections, cannot tell us how long the animal has carried the infection. Thus, we refrain from assuming sequence of infection for statistical tests unless specified by an *a priori* hypothesis from the literature.

2.3.1. Testing for extrinsic drivers of pathogen community composition across the rodent community

We analyzed pathogen community composition across the whole rodent community. We use the term "pathogen community" to refer to the group of viruses and pathogenic bacteria for which we had the means to include, which was not exhaustive; thus measures of diversity are to be considered relative and not absolute. We first estimated pathogen community richness using the Shannon diversity index (alpha diversity) considering pathogenic bacterial OTUs and antiviral antibodies found in each study year, study site, habitat, host species, host sex and host age group (default options (natural logarithm Shannon index) in 'diversity' function from the *vegan* package). We evaluated a linear regression model using analysis of deviance ('lm' and 'drop1' functions from the basic *stats* package) to test for significance of differences in pathogen diversity due to the fixed factors listed above after first correcting for all 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 group factor levels that were not significantly different. The impact of host species diversity (Shannon diversity with Chao's estimator correction using 'Shannon' in package *entropart*) on pathogen species diversity was tested for by correlation ('cor.test', package *stats*) across each year x site x habitat community.

We next tested for differences in pathogen community composition (beta diversity) between host species, habitats, study sites, years, age groups, and sexes by applying a permutational multivariate analysis of variance (PERMANOVA) on a Bray-Curtis dissimilarity matrix ('adonis2' function in

jessie abbate 2/5/21 13:33
Deleted: -

jessie abbate 12/3/20 19:24
Deleted: regroup significantly distinct factor levels

jessie abbate 1/31/21 16:51
Deleted:

jessie abbate 2/2/21 11:34
Deleted: .

the *vegan* package). To explore how intrinsic factors (pathogen-pathogen associations) contributed to the structure of the pathogen community, we used multiple correspondence analysis (MCA) to reduce variance in presence/absence of each bacterial pathogen species and antiviral antibody, implemented with the function 'MCA' in the *FactoMinR* package and visualization tools found in the *factoextra* package. This produces a set of quantitative and orthogonal descriptors (dimensions) describing the pathogen community composition, revealing correlated variables. 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) to understand how the variation described by each MCA dimension was influenced by the extrinsic factors.

2.3.2. 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 (non-significant variation in the MCA dimension among host species or non-significant host species identity x explanatory pathogen term in logistic regressions), 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

jessie abbate 2/2/21 10:22
Deleted: We

jessie abbate 2/5/21 13:33
Deleted: -

jessie abbate 2/2/21 10:35
Deleted: d

jessie abbate 2/1/21 00:12
Deleted: a down to

jessie abbate 2/9/21 03:02
Deleted: *Detecting and t*

jessie abbate 2/1/21 16:24
Deleted: marginal pathogen

jessie abbate 2/1/21 16:24
Deleted: host species

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

The benefit of the SCN approach is a relatively high level of statistical power and the ability to identify precisely which combinations of pathogens occur outside the random expectations (Vaumourin *et al*. (2014)). However, the SCN is sensitive to heterogeneity in the data due to extrinsic factors (e.g., host specificity, or structuring in space, time, age or sex), which can both create and mask true associations. A multiple logistic regression (GLM) approach was thus also systematically employed, as it has the benefit of explicitly taking into account potentially confounding extrinsic factors. Binomial exposure (presence/absence of either bacterial infection or antiviral antibodies) to a single pathogen was set as the dependent variable with exposure to the hypothetically associated pathogen(s) treated as independent explanatory variable(s) and extrinsic factors (host sex, host age, study year, study site, and where appropriate, habitat) were specified as covariates using function 'glm' in the *stats* package with a binomial logit link. When the multiple host species were involved, we tested an interaction term (host species identity x explanatory pathogen), and either (if $p < 0.05$) performed separate analyses for each host species or (if $p \geq 0.05$)

jessie abbate 2/6/21 23:01
Formatted: Font:Italic

jessie abbate 2/6/21 23:02
Formatted: Font:Italic

jessie abbate 2/5/21 13:33
Deleted: -

jessie abbate 2/1/21 11:43
Formatted: Font:Italic

jessie abbate 2/1/21 11:44
Formatted: Font:Italic

simply added host species identity as another covariate in the model. When there was no *a priori* assumption concerning timing of exposure (e.g., antiviral antibody presence is more likely to affect current acute bacterial infection than the reverse), the occurrence of each pathogen involved in a 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 additional interaction terms.

jessie abbate 2/5/21 13:34
Deleted: -

jessie abbate 2/6/21 23:03
Deleted: -

jessie abbate 12/3/20 21:21
Deleted: implicated

2.3.3. Evaluating false discovery

Given the large number of significance tests performed on this single dataset, we compiled all relevant p-values (N=77) and applied a Benjamini-Hochberg correction procedure to estimate how many of the significant results may fall within the false discovery zone (using function 'p.adjust' in the *stats* package). Among tests of positively correlated hypotheses (e.g., pairwise tests of intrinsic pathogen-pathogen associations N=20), only one of the p-values from the pair was included in calculating the false discovery rate (selected randomly). SCN analysis results were not included, because they were also expected to be positively correlated with the logistic regressions, and because the method inherently performs correction for multiple tests. Between hypotheses, the data were often composed of different non-overlapping subsets of varying sizes, and sample sizes varied widely with some being very small. Thus, application of this procedure likely indicates a conservative (low) estimate for how many null hypotheses should truly be rejected.

3. Results

3.1. Taxonomic identification and prevalence of pathogens

3.1.1. Viral exposure

The most abundant virus detected was CPXV, with 222 (32.8%) positive sera of the 677 animals tested for anti-CPXV antibodies. 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; $\chi^2 = 119.5$, $df = 6$, $p < 10^{-15}$). 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; $\chi^2 = 19.4$, $df = 6$, $p = 0.0036$). Anti-LCMV antibodies were detected in two *Mi. arvalis* individuals (Figure 1). No animals were 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 Supplemental Materials Table S1). 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

jessie abbate 2/5/21 16:36
Deleted: infecting

jessie abbate 2/5/21 16:36
Deleted:

jessie abbate 2/5/21 16:36
Deleted: ed

jessie abbate 2/18/21 01:52
Deleted: 111

jessie abbate 2/18/21 01:52
Deleted: 1

jessie abbate 2/18/21 01:52
Deleted: 4

jessie abbate 2/18/21 01:54
Deleted: 12

jessie abbate 2/18/21 01:54
Deleted: 7

jessie abbate 2/18/21 01:54
Deleted: 048

jessie abbate 12/3/20 20:13
Deleted: tested

jessie abbate 2/21/21 12:18
Deleted: Supporting Information

jessie abbate 2/1/21 00:15
Deleted: We checked manually for potential chimera that would not have been removed from the dataset by the *Uchime* program implemented in *mothur*.

from analyses because they were only observed in rare host species, because 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.), because we could not 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: *Myco. haemomuris* (Myco1-3,5,7-9), *Myco. coccoides* (Myco4 and Mco6), and "*Candidatus* Myco. ravipulmonis" (Myco10) (Figure S4). In general, these bacterial infections were present in all but 30 of the 332 animals tested (91.0% 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.

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 marginal mean pathogen diversity

jessie abbate 2/1/21 00:24
Deleted: inability to

jessie abbate 2/18/21 02:18
Deleted: 0.4

differed significantly between host species ($F_{5,69} = 6.86$, $p < 10^{-4}$) and habitats ($F_{2,69} = 4.97$, $p = 0.0096$), and it was significantly higher in adults than in juveniles ($F_{1,69} = 21.43$, $p < 10^{-4}$). Pathogen diversity did not, however, differ between study sites, years, or host sexes (Figure 2, Table S3). Post-hoc Tukey tests showed that after correcting for all other factors in the model, meadow habitats had higher diversity of pathogen exposure than forest habitats, and the diversity of

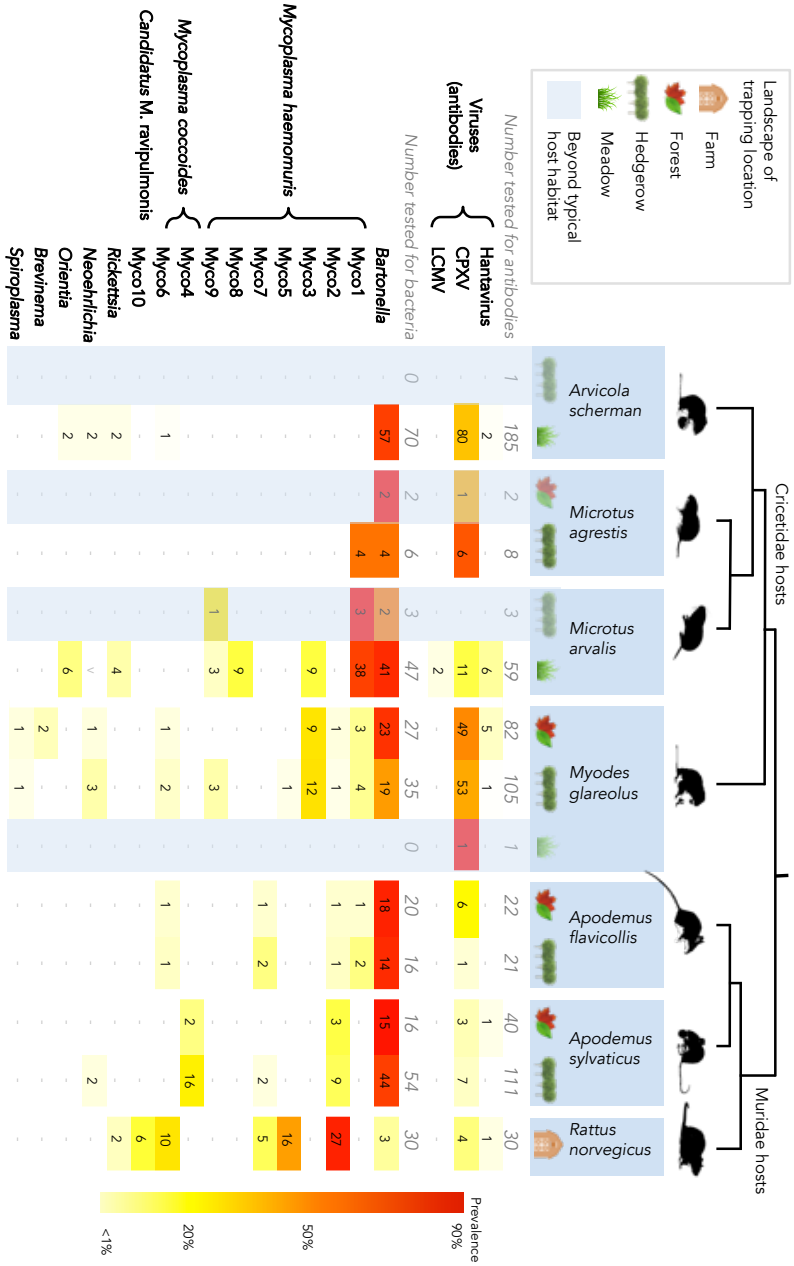


Figure 1. Pathogen occurrence across the rodent species community.

jessie abbate 2/18/21 17:14
Deleted: 78

jessie abbate 2/18/21 17:14
Deleted: 5

jessie abbate 2/18/21 17:15
Deleted: 63

jessie abbate 2/18/21 17:15
Deleted: 005

jessie abbate 1/27/21 01:00
Deleted: and host species ($F_{5,78} = 7.12$, $p < 10^{-4}$)

jessie abbate 2/7/21 01:05
Deleted: . I

jessie abbate 2/18/21 17:15
Deleted: 78

jessie abbate 2/18/21 17:17
Deleted: 32

jessie abbate 2/18/21 17:17
Deleted: 29

jessie abbate 2/18/21 17:15
Deleted: 7

jessie abbate 1/27/21 01:00
Deleted: It

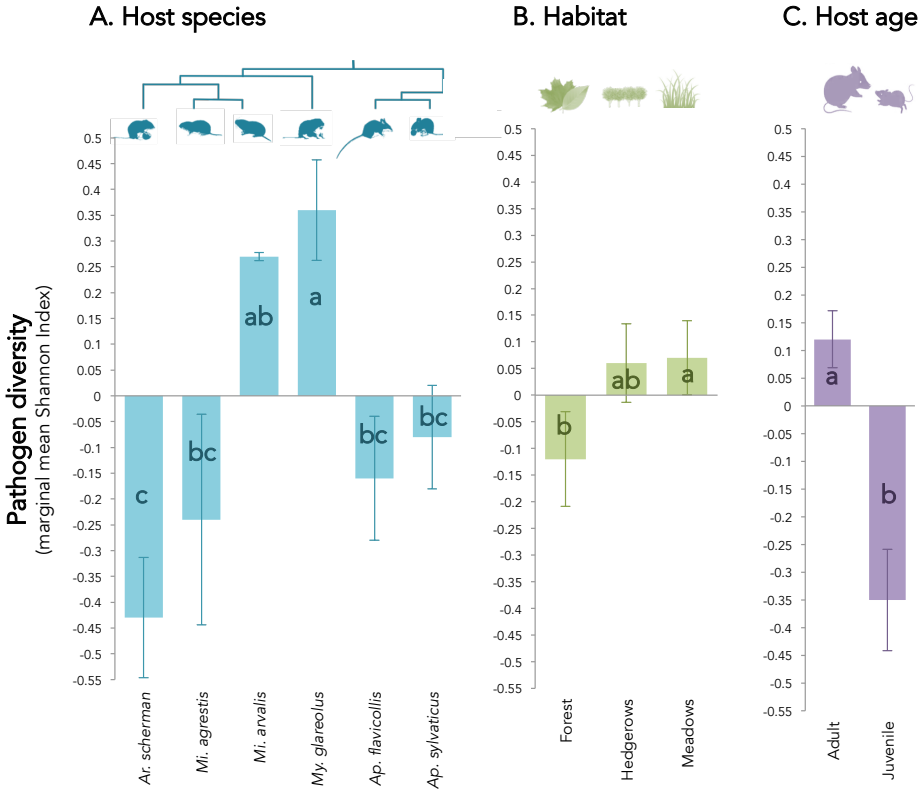
jessie abbate 1/28/21 00:05
Deleted: ... [1]

jessie abbate 2/18/21 17:39
Formatted: Right, Level 1, Indent: First line: 0.5 cm

jessie abbate 2/18/21 17:39
Deleted: .

pathogen communities in host species fell along a continuum between *My. glareolus* (high) and *Ar. scherman* (low) extremes (Figure 2, Table S3). Host species diversity in each community (year x site x habitat) was positively correlated with pathogen diversity (r = 0.62, t = 2.5, df = 10, p = 0.032).

Figure 2: Extrinsic drivers of pathogen diversity in a rodent species community. Differences in Shannon diversity index was tested on marginal means for each factor in the multiple regression model. Different letters signify statistically significant differences at $p < 0.05$, with post-hoc Tukey adjustments for multi-level factors.



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

jessie abbate 1/28/21 00:01
Deleted: Significant d

jessie abbate 1/28/21 00:03
Deleted: were

jessie abbate 1/28/21 00:02
Deleted: residual variance

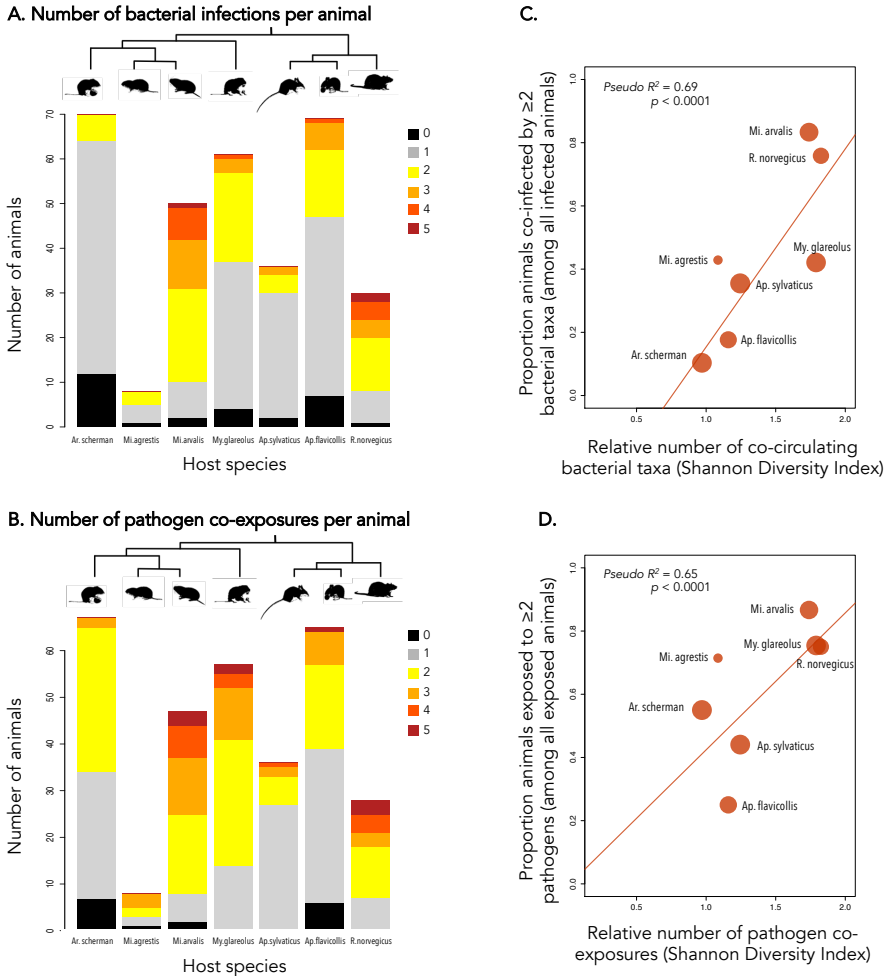
jessie abbate 1/28/21 00:03
Deleted: of

jessie abbate 1/28/21 00:03
Deleted: controlling for all other extrinsic factors

jessie abbate 12/3/20 20:30
Deleted:

community, and that farm habitats were thus in the same high-diversity category as meadow habitats (Table S4).

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



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; analysis of deviance *Pseudo-R^2* = 0.69, $p < 10^{-11}$, 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 antiviral antibodies was considered along with bacterial OTU exposure (Figure 3D; *Pseudo-R^2* = 0.65, $p < 10^{-7}$). 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 S5B): *My. glareolus* co-exposure frequencies were not lower than expected, and both *Apodemus* species had lower than expected co-exposures. Host species diversity in each community did not correlate with bacterial co-infection ($r = 0.33$, $t = 1.11$, df = 10, $p = 0.29$) or pathogen co-exposure ($r = 0.022$, $t = 0.071$, df = 10, $p = 0.94$) frequencies.

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., LCMV), and each host species had a unique combination of co-circulating pathogens (Figure 1). In order to best identify extrinsic and intrinsic factors potentially driving the composition of pathogen communities within the rodent community, we reduced the dataset to limit biases. We excluded *R. norvegicus* individuals due to competing *a priori* hypotheses that host species and habitat would be important factors (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).

jessie abbate 2/20/21 15:50
Deleted: 70

jessie abbate 2/20/21 15:49
Deleted: 0.0001

jessie abbate 2/5/21 13:34
Deleted: -

jessie abbate 1/31/21 23:19
Deleted: were

jessie abbate 2/20/21 15:49
Deleted: 55

jessie abbate 2/20/21 15:49
Deleted: 0.0001

jessie abbate 1/31/21 23:47
Deleted: pathogen

jessie abbate 1/31/21 23:48
Formatted: Font:Italic

jessie abbate 1/31/21 23:48
Formatted: Font:Italic

jessie abbate 1/31/21 23:48
Formatted: Font:Italic

jessie abbate 2/19/21 14:15
Deleted: 31

jessie abbate 1/31/21 23:51
Formatted: Font:Italic

jessie abbate 2/19/21 14:15
Deleted: 99

jessie abbate 1/31/21 23:51
Formatted: Font:Italic

jessie abbate 2/19/21 14:15
Deleted: 2

jessie abbate 2/18/21 17:39
Formatted: Indent: First line: 0.5 cm

jessie abbate 1/28/21 14:21
Deleted: . ... [2]

jessie abbate 2/2/21 10:42
Deleted: factors (host species, host sex and age class, habitat, study site, year)

jessie abbate 2/2/21 10:42
Deleted: performed a single MCA on occurrence data for exposure to both bacteria and viruses followed by logistic regression analyses

jessie abbate 2/2/21 10:48
Deleted: Due to competing *a priori* hypotheses that host species and habitat would be important factors,

jessie abbate 2/2/21 10:48
Deleted: w

Overall, pathogen species composition was significantly structured by host species identity ($F_{5,252}$ = 16.23, p = 0.001) and habitat type ($F_{2,252}$ = 2.51, p = 0.024; Table S5). Out of 14 orthogonal dimensions returned by the MCA, 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 S6). 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 S6). While these first two dimensions varied by host species (Figure S10A) and host age class (Figure S10B), variance in host habitats (Figure S10C) 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 S6).

jessie abbate 2/2/21 11:36
Formatted: Subscript

jessie abbate 2/2/21 11:36
Formatted: Font:Not Italic

jessie abbate 2/2/21 11:36
Formatted: Font:Not Italic

jessie abbate 2/2/21 10:49
Deleted: MCA

jessie abbate 2/2/21 11:46
Deleted: 5

jessie abbate 2/2/21 11:46
Deleted: 5

jessie abbate 2/2/21 11:45
Deleted: 4

jessie abbate 2/2/21 11:45
Deleted: 4

jessie abbate 2/2/21 11:45
Deleted: 4

jessie abbate 2/2/21 11:46
Deleted: 5

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 Myco3 only circulated in two host species (*Mi. arvalis* and

jessie abbate 1/28/21 14:23
Deleted:

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 S6), we also ran a single SCN analysis on the pooled data from adults of both species to improve statistical power (Table S7A). According to this pooled SCN analysis, the three-way co-occurrence of Myco1, Myco3 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 (sitting on the lower bound at zero; p = 0.13; Table S7A, Figure S11). 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 to control for heterogeneous host groups (host species, host sex, study site, habitat, and year sampled) and exposure to the two other pathogens (Table S7B). These models showed that there remained significant unexplained positive associations between hantavirus exposure and Myco1 infection (anti-hantavirus antibodies ~ Myco1: χ^2 = 5.67, p = 0.017; Myco1 ~ anti-hantavirus antibodies: χ^2 = 2.89, p = 0.09) and between hantavirus exposure and Myco3 infection (Myco3 ~ anti-hantavirus antibodies: χ^2 = 12.11, p < 0.001), but that there was no evidence of direct association between Myco1 and Myco3 infections (Myco1 ~ Myco3: χ^2 = 0.07, p = 0.8; Myco3 ~ Myco1: χ^2 < 0.01, p = 0.96; Figure 4A).

jessie abbate 2/2/21 11:46
Deleted: 5

jessie abbate 2/2/21 11:47
Deleted: 6

jessie abbate 12/3/20 19:38
Deleted:

jessie abbate 2/2/21 11:47
Deleted: 6

jessie abbate 2/2/21 11:46
Deleted: 0

jessie abbate 12/3/20 19:36
Deleted: However

jessie abbate 12/3/20 19:39
Deleted: controlling for

jessie abbate 2/2/21 11:47
Deleted: 6

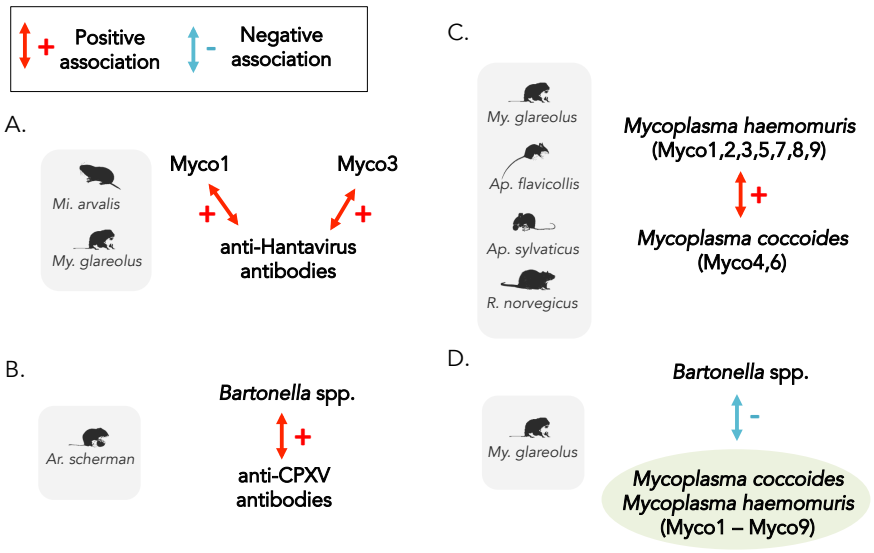
jessie abbate 2/5/21 13:34
Deleted: H

jessie abbate 2/5/21 13:34
Deleted: H

jessie abbate 2/5/21 13:35
Deleted: H

jessie abbate 2/5/21 13:49
Deleted: 5A

Figure 4: 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 (*Myco. haemomuris*) and Myco4 (*Myco. 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 S8A), and after correcting for all extrinsic factors, there remained only a non-significant trend (Myco2 ~ Myco4: $\chi^2 = 2.47$, p = 0.12; Myco4 ~ Myco2: $\chi^2 = 2.47$, p = 0.12; Table S8B) for a positive association between the two OTUs. No additional associations with sufficient variance for statistical tests were clearly suggested by the MCA analysis.

jessie abbate 2/5/21 13:49
Deleted: 5

jessie abbate 1/28/21 14:27
Deleted:

jessie abbate 2/2/21 11:48
Deleted: 7A

jessie abbate 2/2/21 11:47
Deleted: 7

3.3.2. Validation of associations described in the literature

We tested the *a priori* hypothesis that seropositivity to CPXV would be positively associated with *Bartonella* infection, previously detected in *Mi. agrestis* (Telfer *et al.* 2010). The whole dataset was considered as these two pathogens co-circulated in all host species (Figure 1). SCN analyses performed independently for each host species revealed no associations (Table S9A), and the MCA results suggested that pooling data across host species would be inappropriate. After correcting for extrinsic factors using GLM, we found reciprocal evidence for a positive association in *Ar. scherman* hosts (*Bartonella* ~ anti-CPXV antibodies: $\chi^2 = 5.37$, $p = 0.020$; anti-CPXV antibodies ~ *Bartonella*: $\chi^2 = 5.21$, $p = 0.022$; Figure 4B), but not in any other host species (Table S9B). It is of note that there were only eight *Mi. agrestis* individuals, rendering statistical power to test for the association while controlling for extrinsic factors insufficient in this host species where the association was previously described. While prevalence of both pathogens in *Mi. agrestis* was relatively high compared to other host species, one of the two animals without *Bartonella* infection was positive for anti-CPXV antibodies, also precluding evidence for a within-species trend.

We next focused on the potential associations between OTUs identified as belonging to two different species of hemotropic *Mycoplasma, Myco. haemomuris* (HM) and *Myco. coccoides* (HC), within the four host species in which they both circulated (*My. glareolus*, *Ap. flavicollis*, *Ap. sylvaticus*, *R. norvegicus*; Figure 1; Figure S4). We found no significant associations using independent SCN analyses for each host species (Table S10A). However, after controlling for extrinsic factors using GLM, a significant positive association was detected (HM ~ HC: $\chi^2 = 9.5$, $p = 0.0021$; HC ~ HM: $\chi^2 = 9.59$, $p = 0.002$), and did not differ between host species (non-significant interaction term between host species by explanatory pathogen occurrence in each reciprocal model, Table S10B; Figure 4C). We note that only one *R. norvegicus* animal was uninfected with *Myco. haemomuris*, and that animal also had no *Myco. coccoides* infection; thus the trend for the association in this host species was also positive but lacked sufficient variance for independent statistical analysis.

jessie abbate 2/2/21 11:47
Deleted: 8

jessie abbate 2/5/21 13:49
Deleted: 5

jessie abbate 2/2/21 11:48
Deleted: S8B

jessie abbate 2/2/21 11:48
Deleted: 9

jessie abbate 2/4/21 16:29
Deleted: 10.27

jessie abbate 2/4/21 16:29
Deleted: 0013

jessie abbate 2/4/21 16:29
Deleted: 92

jessie abbate 2/4/21 16:29
Deleted: 0016

jessie abbate 2/2/21 11:49
Deleted: 9

jessie abbate 2/5/21 13:49
Deleted: 5

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 S11A), and marginal evidence that any association may differ by host species after correcting for extrinsic factors using GLM (Table S11B). 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 S11B, Figure 4D).

jessie abbate 2/2/21 11:49
Deleted: 10

jessie abbate 2/2/21 11:49
Deleted: 0

jessie abbate 2/2/21 11:49
Deleted: 0

jessie abbate 2/5/21 13:49
Deleted: 5

3.4. Evaluating false discovery

Benjamini-Hochberg correction of p-values from hypothesis tests throughout the study suggested that those above ~0.01 may lie above the false discovery cutoff for statistical significance (Figure S12), and that null hypotheses rejected with smaller p-values have been rejected with confidence.

jessie abbate 2/21/21 11:12
Formatted: Font:Italic, Font color: Text 1

jessie abbate 2/21/21 11:12
Formatted: Font:Italic, Font color: Text 1

4. Discussion

Rodents have long been recognized as important reservoirs of infectious agents, with a high transmission potential to humans and domestic animals (Kruse, Kirkemo & Handeland 2004). 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 and antibodies against at

jessie abbate 2/6/21 06:35
Deleted: (23 OTUs)

least four genera of viruses. Some of these pathogens have already been reported in the study 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)). A previously undetected relative of the putatively pathogenic spirochaete *Brevinema andersonii* that infects short-tailed shrews and white-footed mice in North America (Defosse *et al.* 1995) was among our findings, and TBEV is not known to circulate this far east (Lindquist & Vapalahti 2008). The high prevalence of anti-hantavirus antibodies in *Mi. arvalis* is likely 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 zoonotic pathogens were particularly prevalent: *Orthopoxvirus*, *Bartonella* spp., and *Mycoplasma* spp. The wide range of hosts with anti-*Orthopoxvirus* antibodies 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* spp., 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* spp. 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 across all host species, though surprisingly lower than expected in *Ar. scherman* (Villette *et al.* 2017). These *Mycoplasma* species are also known pathogens of humans and rodents (Harwick, Kalmanson & Guze 1972; Baker 1998). Here, we found to two distinct hemotropic *Mycoplasma* species (*Myco. haemomuris* and *Myco.*

jessie abbate 2/6/21 06:36
Deleted: circulating in the areas examined

jessie abbate 2/6/21 06:36
Deleted: this

jessie abbate 2/6/21 06:37
Deleted: ;

jessie abbate 2/6/21 15:27
Deleted: Several previously unseen agents were also detected in our study, such as Ljungan virus, a picornavirus found in other European rodent populations (e.g., Salisbury *et al.* 2014), and a

jessie abbate 2/6/21 15:29
Deleted: In addition, we detected a r

jessie abbate 2/6/21 15:29
Deleted: elatively

jessie abbate 2/6/21 15:29
Deleted: ,

jessie abbate 2/6/21 16:30
Deleted: Three pathogens were highly prevalent

jessie abbate 2/6/21 16:33
Deleted: Anti

jessie abbate 2/6/21 16:33
Deleted: were detected in all seven focal rodent species, with prevalence levels reaching up to 70%. This

jessie abbate 2/6/21 13:34
Formatted: Font:Not Italic, English (UK)

jessie abbate 2/6/21 16:35
Deleted: 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

jessie abbate 2/6/21 16:37
Deleted: ,

jessie abbate 2/6/21 16:37
Deleted: 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

jessie abbate 2/6/21 16:38
Deleted: from Eastern France

jessie abbate 2/6/21 16:29
Deleted: 15 different OTUs corresponding

coccoides) and the pneumotropic *Mycoplasma* species *Myco. pulmonis* and "*Candidatus* Myco. ravipulmonis". The former two are both hemotropic mycoplasmas responsible for vector-transmitted infectious anaemia of wild mice, rats, and other rodent species (Neimark *et al.* 2001, 2005; Messick 2004). In contrast, *Myco. pulmonis* and "*Candidatus* Myco. ravipulmonis" cause respiratory infections, are more closely related to other pneumotropic mycoplasmas, and "*Candidatus* Myco. ravipulmonis" has only ever before been described in laboratory mice (formerly termed Grey Lung virus (Andrews & Glover 1945; Neimark, Mitchelmore & Leach 1998; Graham & Schoeb 2011; Piasecki, Chrzastek & Kasprzykowska 2017)).

Our results also corroborated the status of hyper-reservoir (more than two zoonotic pathogens carried by a reservoir species) for all seven of the focal rodent species studied here (Han *et al.* 2015). Even the rare host species *Mi. subterraneous* also carried two potentially zoonotic pathogens (*Bartonella* spp. and *Brevinema* spp.; Appendix 1). Overall, we found a high variability in the number of pathogens circulating in each species despite correction for sampling effort, with low levels observed in *Apodemus* species and *Arvicola scherman*, and high levels detected in *Mi. arvalis, My. glareolus*, and *R. norvegicus*. While physiology, genetics, and behavior can contribute to the number of pathogen species able to infect a given host species, larger geographic range size is highly correlated with higher pathogen species diversity (Morand 2015); this explanation matches the pattern among hosts in the communities sampled here (i.e., *Ar. scherman* and *Apodemus* spp. have small geographic ranges compared to those of *Mi. arvalis*, My. *glareolus*, and *R. norvegicus*).

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

jessie abbate 12/3/20 19:59
Deleted: However

jessie abbate 12/3/20 20:00
Deleted: as spontaneously occurring in laboratory mice and rats exposed to unknown microbes of other animals and humans via

jessie abbate 12/3/20 20:02
Deleted: passaging experiments

jessie abbate 2/7/21 11:05
Deleted: ;

jessie abbate 2/6/21 16:41
Deleted: Lastly, our data

jessie abbate 2/6/21 16:45
Deleted: , with the addition of

jessie abbate 2/6/21 16:42
Deleted:

jessie abbate 2/6/21 16:41
Deleted: that

jessie abbate 2/6/21 16:42
Deleted: we also found to

jessie abbate 2/6/21 16:42
Deleted: y

jessie abbate 2/6/21 21:53
Formatted: Font:Not Italic

found across both meadows and hedgerows. Instead, we found that more diverse host species communities hosted more diverse pathogen communities. However, the implications of that result are complex because while exposure to diverse (i.e., potentially novel) pathogens is a risk for disease emergence, diverse host species communities are thought to keep individual pathogen prevalence low due to the dilution effect – which should limit risk of zoonoses (Keesing *et al.* 2010).

The search for factors that drive parasite species richness, diversity and community composition has been at the core of numerous studies (Poulin 1995; Poulin & Morand 2000; Nunn *et al.* 2003; Mouillot *et al.* 2005; Krasnov *et al.* 2010; Sallinen *et al.* 2020). Here, we emphasized that both pathogen diversity and community composition was mainly structured by host species identity, despite both shared habitats and shared pathogen taxa. Pathogen beta diversity was also structured by habitat, which could result from particular environmental suitability (e.g., for vectors) or opportunities for cross-species transmission. We found no evidence that any specific pathogen-pathogen associations were likely to be as important as host species identity in determining pathogen distributions across the community of rodents. The strong influence of host characteristics (Cohen *et al.* 2015) and host species identity (Dallas *et al.* 2019) on pathogen community composition has recently been described in comparison to intrinsic pathogen-pathogen associations in other rodents. Moreover, the pathogen community composition provided a unique signature for each rodent species, even among those most closely related (e.g., *Ap. flavicolis* and *Ap. sylvaticus*). This result is in line with the conclusions of meta-analyses showing that phylogeny, over other host traits, has a minimal impact on pathogen diversity in rodent species (Luis *et al.* 2013; Guy *et al.* 2019).

The importance of host species identity in shaping pathogen community composition may not stem from strict host-pathogen specificity, as most pathogens were found to infect multiple host species – a broad result echoed across animal communities (Taylor *et al.* 2001; Woolhouse, Taylor & Haydon 2001; Cleaveland, Laurenson & Taylor 2001; Pedersen *et al.* 2005; Streicker *et al.*

jessie abbate 2/6/21 13:44
Deleted: Using multiple correspondence analysis (MCA) together with logistic regression and the novel computational method of association screening (SCN) analysis, w

jessie abbate 2/7/21 00:37
Deleted: of

jessie abbate 2/6/21 13:46
Deleted: recent

jessie abbate 2/6/21 13:46
Deleted: d

2013). However, we might be cautious as more precise molecular analyses are necessary to test whether different species of a bacteria genus or divergent populations of the same bacteria species may circulate independently in different rodent host species, with little or no transmission. For example, two genera seemed to be largely shared among the rodent species studied here, *Bartonella* and *Mycoplasma*. But previous studies have shown strong host-specificity when considering the genetic variants of *Bartonella* (Buffet *et al.* 2013; Withenshaw *et al.* 2016; Brook *et al.* 2017) Evidence in the literature for host specificity of *Mycoplasma* species has led to a mix of conclusions (Pitcher & Nicholas 2005), as cases of cross-species transmission are commonly reported – particularly in humans – despite a general consensus that most species are highly host-specific. We found that some *Mycoplasma* taxa were dominant contributors to prevalence in a single host species, and that when shared, they were shared with just a few other specific host species. Rare infections in unexpected host species (e.g., Myco6 in *Ar. scherman* and Myco1 in *Ap. flavicollis*) were represented by fewer sequence reads compared to positive samples in host species where they 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 *Myco. 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* (sister to all other sampled Cricetidae 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.

Concurrent exposure to multiple pathogens within individuals was also frequent, as high as 89 % (in *Mi. arvalis* hosts), in line with recent studies that have shown that co-infections by multiple pathogens are common in natural populations (e.g., in mammals, birds, amphibians, ticks, humans (Telfer *et al.* 2010; Griffiths *et al.* 2011; Moutailler *et al.* 2016; Clark *et al.* 2016; Stutz *et al.*

jessie abbate 12/3/20 19:53
Deleted: detected

jessie abbate 12/3/20 19:53
Deleted: ,

jessie abbate 2/6/21 23:21
Deleted: the most basal member of the

jessie abbate 2/6/21 23:21
Deleted: included

jessie abbate 2/6/21 22:11
Deleted: 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)).

jessie abbate 2/6/21 16:49
Deleted: ~~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

jessie abbate 2/6/21 15:44
Formatted: Strikethrough

jessie abbate 1/31/21 23:47
Formatted: French

2018)). Variation in the frequency of pathogen co-exposure was highly correlated to the diversity of pathogens circulating in each host species, suggesting the dominance of a random process of pathogen exposure for each individual. However, there were a few intriguing outliers: *My. glareolus* hosts were less co-infected than expected based on diversity of bacterial taxa, but not when viral antibodies were included; conversely, *Ar. scherman* hosts were more co-exposed when viruses were considered, but not when only bacteria were considered; and *Mi. arvalis* hosts had consistently higher proportions of co-exposures whether viruses were or were not considered along with bacteria. The non-random grouping of pathogen exposures within individuals (as in *Mi. arvalis*) may result from heterogeneity in extrinsic transmission, environmental, or susceptibility factors (Cattadori *et al.* 2006; Swanson *et al.* 2006; Beldomenico *et al.* 2008; Fenton, Viney & Lello 2010; Beldomenico & Begon 2010) or from intrinsic interactions between pathogens (e.g., facilitation mediated by hosts immune response). Differences in the pattern of co-exposure frequencies when including or excluding antiviral antibodies (as with *My. glareolus* and *Ar. scherman*) could result from different mechanisms (e.g., bacterial manipulation of innate immunity (Diacovich & Gorvel 2010)) affecting pathogen community assemblage. However, a lack of deviance from the expected co-exposure frequency does not exclude the possibility that both extrinsic and intrinsic processes may be occurring.

We found evidence in support of three previously identified pathogen-pathogen associations (positive association between *Myco. haemomuris* and *Myco. coccoides* infections; positive association between *Bartonella* spp. infection and the presence of anti-CPXV antibodies; negative association between *Bartonella* spp. and hemotropic *Mycoplasma* spp. infections), and characterized one set of associations not previously described (positive associations between the presence of anti-hantavirus antibodies and infections by two specific *Myco. haemomuris* OTUs) – each in a unique subset of host species. *Mycoplasma* spp. blood infections are likely transmitted through bites of blood-sucking arthropod vectors (Volokhov *et al.* 2017), meaning vectors could prefer some individuals over others. Positive associations detected between *Myco. haemomuris* and

jessie abbate 2/6/21 16:50
Deleted: Here, we also observed high variability in the percentage of individuals with evidence of multiple current or prior infections between the rodent species investigated. This v

jessie abbate 2/6/21 16:51
Deleted: ~~Here, we tested for pathogen-pathogen associations suggested by multiple correspondence analysis (MCA) of the structure of pathogen exposures in this specific rodent community, as well as those previously described in the literature. We used multiple logistic regression analyses (via analysis of deviance on generalized linear models (GLMs)) to determine whether pathogen-pathogen associations were present after extrinsic factors creating heterogeneity among hosts were taken into account.~~

jessie abbate 2/6/21 15:46
Formatted: Strikethrough

jessie abbate 2/6/21 19:52
Deleted: . ... [3]

jessie abbate 2/6/21 19:47
Deleted: is not yet well understood, but given a lack of support for density-dependent transmission and high incidence of spill-over events, it is

jessie abbate 2/6/21 19:48
Deleted: that these bacteria are

jessie abbate 2/6/21 19:51
Deleted: The p

Myco. coccoides could also result from similarities in rodent susceptibility. Indeed *Mycoplasma* spp. 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 *Myco. haemomuris* OTUs (Myco1 and Myco3) and anti-hantavirus antibodies may similarly be explained by the chronic nature of both *Mycoplasma* spp. 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 occurred (*Microtus arvalis* and *Myodes glareolus*), consistent with the generality of association between *Mycoplasma* species across host taxa detailed above, suggesting the intrinsic ecology of these pathogens contributes to shaping variation in the pathogen community. Curiously, we found no evidence for direct associations between OTUs of the same *Mycoplasma* species, thus facilitation interactions are unlikely to explain the high diversity of *Mycoplasma* taxa both within and between host species.

Infections by *Bartonella* species are also known to often result in subclinical and persistent bacteremia in mammals, including rodents (Birtles *et al.* 2001; Kosoy *et al.* 2004). The positive association detected in *Ar. scherman* between *Bartonella* spp. and anti-CPXV antibodies might therefore be explained by, for example, joint accumulation of both chronic bacterial infections and long-lived antiviral antibodies used to test for prior exposure to relatively short-lived CPXV infections. However, if the same processes governing association of the chronic infections described

jessie abbate 1/31/21 22:45
Deleted: independent of host species identity

jessie abbate 12/3/20 19:30
Deleted: cowpox virus

jessie abbate 12/3/20 19:29
Deleted: virus

above were at play here, we would have expected to find both pathogens implicated in positive associations (i) with other chronic infections, and (ii) across host species given their ubiquitous prevalence. While the failure to recover the association in *Mi. agrestis* (previously described (Telfer *et al.* 2010)) was likely due to low statistical power, the lack of a general pattern across other host species despite adequate sampling suggests a more specific, and potentially immune-mediated, ecological process between these two pathogens. Indeed, pox virus infections, including CPXV, have been shown to induce immunomodulation that increases host susceptibility to other parasites (Johnston & McFadden 2003). These interactions could be of variable intensities according to the rodent species considered, due to potential differences in impacts of CPXV infection on immunity across host species, or to the influence of other infections not examined here on host immune responses during pox infections (e.g., helminths (Cattadori, Albert & Boag 2007), protozoa (Telfer *et al.* 2010)). Furthermore, *Bartonella* spp. infection was negatively associated with *Mycoplasma* spp. infections in *My. glareolus*, corroborating negative interactions reported in co-infection experiments in gerbils (Eidelman *et al.* 2019). This association may therefore originate from an interaction mediated by specific (immune) genetic features of *My. glareolus*, and not ecological conditions as proposed by Eidelman et al. (2019). The antagonistic and host-specific nature of this association lends further support to the interpretation that *Bartonella* spp. infections do not behave in similar ways to other chronic infections in the community. However, few studies have investigated the robustness of within-host interactions across different host species (e.g., (Lello *et al.* 2018)), and this question deserves further investigation.

Our results suggest that intrinsic ecological interactions could help shape the composition of the pathogen community within hosts. However, this suggestion provides only a hypothesis that requires further investigation. Interpretation of associations can be misleading, as they may arise from unmeasured co-factors such as exposure to shared transmission routes, and may even run counter to the underlying ecological process (Fenton *et al.* 2014). The associations we found here were not visible (or even misleading, in the case of a 3-way interaction between hantavirus, Myco1

jessie abbate 12/3/20 19:32
Deleted: where it was

jessie abbate 12/3/20 19:29
Deleted: revealed

jessie abbate 2/6/21 19:53
Deleted: -

jessie abbate 2/6/21 15:48
Deleted: to establish its provenance

jessie abbate 2/6/21 15:52
Deleted: We noted that t

jessie abbate 2/6/21 15:49
Deleted: were considered properly characterized only once extrinsic factors had been taken into account using multiple logistic regression analyses, and

and Myco3), for instance, when ignoring extrinsic factors using the SCN analysis, despite the increased statistical power it offered. Evidence for interactions between pathogens within hosts initially came from laboratory studies (e.g., in the development of vaccines, reviewed in (Casadevall & Pirofski 2000)), and until recently, many studies conducted in the wild could not detect such interactions (e.g., (Behnke 2008)). Developments of statistical approaches have contributed to improve sampling designs and analyses, in particular by better controlling for confounding factors, enabling the detection of associations resulting from these within-host interactions (e.g., (Lello *et al.* 2004; Telfer *et al.* 2010)). However, it is unlikely any statistical approach can ever solve the problem of an unmeasured explanatory variable. Experiments conducted in semi-controlled environments have been used to confirm the importance of interactions suggested by the associations (e.g., (Knowles *et al.* 2013)). Both facilitation mediated by immune responses (e.g., (Ezenwa *et al.* 2010)) and competition mediated 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 ours. For instance, they can not provide information about the sequence or duration 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

jessie abbate 2/6/21 15:50
Deleted: these factors

jessie abbate 2/6/21 15:53
Deleted: we have presented here

jessie abbate 2/6/21 15:53
Deleted: and

jessie abbate 2/6/21 15:53
Deleted: timing

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. This was due mainly to the fact that 16S data cannot often distinguish between pathogenic and commensal taxa of many such genera. We know that, for instance, *Helicobacter* species are naturally found in the digestive tract, but can also cause pathogenic infections. 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; Rosshart *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 or other viruses, which may explain or alter the associations we were able to detect. Furthermore, the evaluation of diversity measures (e.g., Shannon diversity index) based only on a selection of taxa violates the assumption that all species are represented in the sample; thus, patterns of diversity could also be influenced by missing data. 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).

jessie abbate 2/6/21 15:58
Deleted: We also know that p

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 undescribed 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 rodent 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.

jessie abbate 12/3/20 19:28
Deleted: -

jessie abbate 2/21/21 11:12
Deleted: s

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, Sylvain Piry, Alexandre Dehne-Garcia and Marie Pagès for their help with the bioinformatic analysis, and Gael Kergoat for helpful comments and proofreading.

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 (iv) filtered occurrence table and (v) scripts and data files for statistical analyses. Items iii-v are also provided in Supplemental Materials Appendix 2 to directly accompany this publication.

jessie abbate 2/7/21 02:54
Deleted: and

jessie abbate 2/21/21 11:01
Deleted: and

jessie abbate 2/21/21 11:01
Deleted: .

jessie abbate 2/21/21 11:01
Deleted: S

jessie abbate 2/21/21 11:02
Deleted: included

Jessie Abbate 2/21/21 13:04
Deleted: 1

1080 Table 1. BLAST search results for OTUs suspected of belonging to pathogenic genera.
1081

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

1082

1083 Table 1 (continued). BLAST search results for OTUs suspected of belonging to pathogenic genera.
1084

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

1085

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.

Beechler, B.R., Manore, C.A., Reininghaus, B., O'Neal, D., Gorsich, E.E., Ezenwa, V.O. & Jolles, A.E. (2015) Enemies and turncoats: Bovine tuberculosis exposes pathogenic potential of rift valley fever virus in a common host, African buffalo (*Syncerus caffer*). *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20142942.

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,

1112 C.M., Vicary, L. & Baxby, D. (1997) Cowpox in British voles and mice. *Journal of*
1113 *Comparative Pathology*, **116**, 35–44.
1114 Birtles, R.J., Hazel, S.M., Bennett, M., Bown, K., Raoult, D. & Begon, M. (2001) Longitudinal
1115 monitoring of the dynamics of infections due to *Bartonella* species in UK woodland rodents.
1116 *Epidemiology and infection*, **126**, 323–329.
1117 Bordes, F., Caron, A., Blasdell, K., de Garine-Wichatitsky, M. & Morand, S. (2017) Forecasting
1118 potential emergence of zoonotic diseases in South-East Asia: network analysis identifies key
1119 rodent hosts. *Journal of Applied Ecology*, **54**, 691–700.
1120 Bordes, F., Herbreteau, V., Dupuy, S., Chaval, Y., Tran, A. & Morand, S. (2013) The diversity of
1121 microparasites of rodents: a comparative analysis that helps in identifying rodent-borne rich
1122 habitats in Southeast Asia. *Infection ecology & epidemiology*, **3**.
1123 Breitschwerdt, E.B. (2014) Bartonellosis: One health perspectives for an emerging infectious
1124 disease. *ILAR Journal*, **55**, 46–58.
1125 Breitschwerdt, E.B. & Kordick, D.L. (2000) Bartonella infection in animals: Carriership, reservoir
1126 potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbiology*
1127 *Reviews*, **13**, 428–438.
1128 Brook, C.E., Bai, Y., Yu, E.O., Ranaivoson, H.C., Shin, H., Dobson, A.P., Metcalf, C.J.E., Kosoy,
1129 M.Y. & Dittmar, K. (2017) Elucidating transmission dynamics and host-parasite-vector
1130 relationships for rodent-borne Bartonella spp. in Madagascar. *Epidemics*, **20**, 56–66.
1131 Brown, A.F. (1986) Evidence for density-dependent establishment and survival of *Pomphorhynchus*
1132 *laevis* (Müller, 1776) (*Acanthocephala*) in laboratory-infected *Salmo gairdneri* Richardson and
1133 its bearing on wild populations in *Leuciscus cephalus* (L.). *Journal of Fish Biology*, **28**, 659–
1134 669.
1135 Budischak, S.A., Sakamoto, K., Megow, L.C., Cummings, K.R., Urban, J.F. & Ezenwa, V.O.
1136 (2015) Resource limitation alters the consequences of co-infection for both hosts and parasites.
1137 *International Journal for Parasitology*, **45**, 455–463.

1138 Budischak, S.A., Wiria, A.E., Hamid, F., Wammes, L.J., Kaisar, M.M.M., van Lieshout, L.,
1139 Sartono, E., Supali, T., Yazdanbakhsh, M. & Graham, A.L. (2018) Competing for blood: the
1140 ecology of parasite resource competition in human malaria–helminth co-infections. *Ecology*
1141 *Letters*, **21**, 536–545.
1142 Buffet, J.-P., Pisanu, B., Brisse, S., Roussel, S., Félix, B., Halos, L., Chapuis, J.-L. & Vayssier-
1143 Taussat, M. (2013) Deciphering *Bartonella* Diversity, Recombination, and Host Specificity in
1144 a Rodent Community. *PLOS ONE*, **8**, e68956.
1145 Burthe, S., Telfer, S., Lambin, X., Bennett, M., Carslake, D., Smith, A. & Begon, M. (2006)
1146 Cowpox virus infection in natural field vole *Microtus agrestis* populations: delayed density
1147 dependence and individual risk. *Journal of Animal Ecology*, **75**, 1416–1425.
1148 Carvalho-Pereira, T.S.A., Souza, F.N., Santos, L.R. do N., Pedra, G.G., Minter, A., Bahiense, T.C.,
1149 Reis, M.G., Ko, A.I., Childs, J.E., da Silva, E.M., Costa, F. & Begon, M. (2019) Coinfection
1150 modifies carriage of enzootic and zoonotic parasites in Norway rats from an urban slum.
1151 *Ecosphere*, **10**, e02887.
1152 Casadevall, A. & Pirofski, L.A. (2000) Host-pathogen interactions: basic concepts of microbial
1153 commensalism, colonization, infection, and disease. *Infection and immunity*, **68**, 6511–6518.
1154 Cattadori, I.M., Albert, R. & Boag, B. (2007) Variation in host susceptibility and infectiousness
1155 generated by co-infection: the myxoma–*Trichostrongylus retortaeformis* case in wild rabbits.
1156 *Journal of The Royal Society Interface*, **4**, 831–840.
1157 Cattadori, I.M., Boag, B. & Hudson, P.J. (2008) Parasite co-infection and interaction as drivers of
1158 host heterogeneity. *International Journal for Parasitology*, **38**, 371–380.
1159 Cattadori, I.M., Haukisalmi, V., Henttonen, H. & Hudson, P.J. (2006) Transmission ecology and the
1160 structure of parasite communities in small mammals BT - Micromammals and
1161 Macroparasites: From Evolutionary Ecology to Management. (eds S. Morand, B.R. Krasnov &
1162 R. Poulin), pp. 349–369. Springer Japan, Tokyo.
1163 Charbonnel, N., Deter, J., Chaval, Y., Laakkonen, J., Henttonen, H., Voutilainen, L., Vapalahti, O.,

Vaheri, A., Morand, S. & Cosson, J.-F. (2008) Serological Evidence of Viruses Naturally Associated with the Montane Water Vole (Arvicola scherman) in Eastern France. *Vector-Borne and Zoonotic Diseases*, **8**, 763–768.

Charbonnel, N., Pagès, M., Sironen, T., Henttonen, H., Vapalahti, O., Mustonen, J. & Vaheri, A. (2014) Immunogenetic factors affecting susceptibility of humans and rodents to hantaviruses and the clinical course of hantaviral disease in humans. *Viruses*, **6**, 2214–2241.

Claesson, M.J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P. & O'Toole, P.W. (2010) Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Research*, **38**, e200-213.

Clark, N.J., Wells, K., Dimitrov, D. & Clegg, S.M. (2016) Co-infections and environmental conditions drive the distributions of blood parasites in wild birds. *Journal of Animal Ecology*, **85**, 1461–1470.

Cleaveland, S., Laurenson, M.K. & Taylor, L.H. (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **356**, 991–999.

Cohen, C., Einav, M. & Hawlena, H. (2015) Path analyses of cross-sectional and longitudinal data suggest that variability in natural communities of blood-associated parasites is derived from host characteristics and not interspecific interactions. *Parasites & vectors*, **8**, 429.

Cohen, C., Shemesh, M., Garrido, M., Messika, I., Einav, M., Khokhlova, I., Tasker, S. & Hawlena, H. (2018) Haemoplasmas in wild rodents: Routes of transmission and infection dynamics. *Molecular Ecology*, **27**, 3714–3726.

Cougoul, A., Bailly, X., Vourc'h, G. & Gasqui, P. (2019) Rarity of microbial species: In search of reliable associations. *PLOS ONE*, **14**, e0200458.

Cox, F.E.G. (2001) Concomitant infections , parasites and immune responses. *Parasitology*, **122**, S23–S38.

1190 Dallas, T.A., Laine, A.-L. & Ovaskainen, O. (2019) Detecting parasite associations within multi-
1191 species host and parasite communities. *Proceedings of the Royal Society B: Biological*
1192 *Sciences*, **286**, 20191109.
1193 Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000) Emerging Infectious Diseases of Wildlife -
1194 Threats to Biodiversity and Human Health. *Science*, **287**, 443–449.
1195 Davies, T.J. & Pedersen, A.B. (2008) Phylogeny and geography predict pathogen community
1196 similarity in wild primates and humans. *Proceedings. Biological sciences / The Royal Society*,
1197 **275**, 1695–701.
1198 Davis, S., Calvet, E. & Leirs, H. (2005) Fluctuating Rodent Populations and Risk to Humans from
1199 Rodent-Borne Zoonoses. *Vector-Borne and Zoonotic Diseases*, **5**, 305–314.
1200 Defosse, D.L., Johnson, R.C., Paster, B.J., Dewhirst, F.E. & Fraser, G.J. (1995) *Brevinema*
1201 *andersonii* gen. nov., sp. nov., an infectious spirochete isolated from the short-tailed shrew
1202 (*Blarina brevicauda*) and the white-footed mouse (*Peromyscus leucopus*). *International*
1203 *journal of systematic bacteriology*, **45**, 78–84.
1204 Deter, J., Chaval, Y., Galan, M., Gauffre, B., Morand, S., Henttonen, H., Laakkonen, J.,
1205 Voutilainen, L., Charbonnel, N. & Cosson, J.-F. (2007) Kinship, dispersal and hantavirus
1206 transmission in bank and common voles. *Archives of Virology*, **153**, 435.
1207 Diacovich, L. & Gorvel, J.-P. (2010) Bacterial manipulation of innate immunity to promote
1208 infection. *Nature Reviews Microbiology*, **8**, 117–128.
1209 Diagne, C., Galan, M., Tamisier, L., d'Ambrosio, J., Dalecky, A., Bâ, K., Kane, M., Niang, Y.,
1210 Diallo, M., Sow, A., Gauthier, P., Tatard, C., Loiseau, A., Piry, S., Sembène, M., Cosson, J.-F.,
1211 Charbonnel, N. & Brouat, C. (2017) Ecological and sanitary impacts of bacterial communities
1212 associated to biological invasions in African commensal rodent communities. *Scientific*
1213 *reports*, **7**, 14995.
1214 Eidelman, A., Cohen, C., Navarro-Castilla, Á., Filler, S., Gutiérrez, R., Bar-Shira, E., Shahar, N.,
1215 Garrido, M., Halle, S., Romach, Y., Barja, I., Tasker, S., Harrus, S., Friedman, A. & Hawlena,

H. (2019) The dynamics between limited-term and lifelong coinfecting bacterial parasites in wild rodent hosts. *The Journal of Experimental Biology*, **222**, jeb203562.

Essbauer, S., Pfeffer, M. & Meyer, H. (2010) Zoonotic poxviruses. *Veterinary Microbiology*, **140**, 229–236.

Ezenwa, V.O., Etienne, R.S., Luikart, G., Beja-Pereira, A. & Jolles, A.E. (2010) Hidden Consequences of Living in a Wormy World: Nematode-Induced Immune Suppression Facilitates Tuberculosis Invasion in African Buffalo. *The American Naturalist*, **176**, 613–624.

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

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

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

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

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

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

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

mSystems, **1**, e00032-16.

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

Gaulke, C.A., Martins, M.L., Watral, V.G., Humphreys, I.R., Spagnoli, S.T., Kent, M.L. & Sharpton, T.J. (2019) A longitudinal assessment of host-microbe-parasite interactions resolves the zebrafish gut microbiome's link to Pseudocapillaria tomentosa infection and pathology. *Microbiome*, **7**, 10.

Gorsich, E.E., Ezenwa, V.O. & Jolles, A.E. (2014) Nematode-coccidia parasite co-infections in African buffalo: Epidemiology and associations with host condition and pregnancy. *International Journal for Parasitology: Parasites and Wildlife*, **3**, 124–134.

Gotteland, C., Chaval, Y., Villena, I., Galan, M., Geers, R., Aubert, D., Poulle, M.-L., Charbonnel, N. & Gilot-Fromont, E. (2014) Species or local environment, what determines the infection of rodents by *Toxoplasma gondii*? *Parasitology*, **141**, 259–268.

Graham, J.E. & Schoeb, T.R. (2011) *Mycoplasma pulmonis* in Rats. *Journal of Exotic Pet Medicine*, **20**, 270–276.

Griffiths, E.C., Pedersen, A.B., Fenton, A., Petchey, O.L. & Sheffield, S. (2011) The nature and consequences of coinfection in humans. *The Journal of infection*, **63**, 200–6.

Guivier, E., Galan, M., Henttonen, H., Cosson, J.-F. & Charbonnel, N. (2014) Landscape features and helminth co-infection shape bank vole immunoheterogeneity, with consequences for Puumala virus epidemiology. *Heredity*, **112**, 274–281.

Guy, L., Nystedt, B., Toft, C., Zaremba-Niedzwiedzka, K., Berglund, E.C., Granberg, F., Näslund, K., Eriksson, A.S. & Andersson, S.G.E. (2013) A Gene Transfer Agent and a Dynamic Repertoire of Secretion Systems Hold the Keys to the Explosive Radiation of the Emerging

Pathogen *Bartonella*. *PLoS Genetics*, **9**, e1003393.

Guy, C., Thiagavel, J., Mideo, N. & Ratcliffe, J.M. (2019) Phylogeny matters: revisiting 'a comparison of bats and rodents as reservoirs of zoonotic viruses.' *Royal Society Open Science*, **6**, 181182.

Han, B.A., Schmidt, J.P., Bowden, S.E. & Drake, J.M. (2015) Rodent reservoirs of future zoonotic diseases. *Proceedings of the National Academy of Sciences*, **112**, 7039–7044.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. & Samuel, M.D. (2002) Climate warming and disease risks for terrestrial and marine biota. *Science*, **296**, 2158–2162.

Harwick, H.J., Kalmanson, G.M. & Guze, L.B. (1972) Human diseases associated with Mycoplasmas. *California Medicine*, **116**, 1–7.

Iralu, J., Bai, Y., Crook, L., Tempest, B., Simpson, G., McKenzie, T. & Koster, F. (2006) Rodent-associated *Bartonella* febrile illness, southwestern United States. *Emerging Infectious Diseases*, **12**, 1081–1086.

Johnston, J.B. & McFadden, G. (2003) Poxvirus Immunomodulatory Strategies: Current Perspectives. *Journal of Virology*, **77**, 6093 LP – 6100.

Jolles, A.E., Ezenwa, V.O., Etienne, R.S., Turner, W.C. & Olff, H. (2008) Interactions between macroparasites and microparasites drive infection patterns in free-ranging african buffalo. *Ecology*, **89**, 2239–2250.

Jones, K.E., Patel, N.G., Levy, M. a, Storeygard, A., Balk, D., Gittleman, J.L. & Daszak, P. (2008) Global trends in emerging infectious diseases. *Nature*, **451**, 990–3.

Kallio-Kokko, H., Laakkonen, J., Rizzoli, A., Tagliapietra, V., Cattadori, I., Perkins, S.E., Hudson, P.J., Cristofolini, A., Versini, W., Vapalahti, O., Vaheri, A. & Henttonen, H. (2006) Hantavirus and arenavirus antibody prevalence in rodents and humans in Trentino, Northern Italy. *Epidemiology and Infection*, **134**, 830.

Kedem, H., Cohen, C., Messika, I., Einav, M., Pilosof, S. & Hawlena, H. (2014) Multiple effects of

host-species diversity on coexisting host-specific and host-opportunistic microbes. *Ecology*, **95**, 1173–1183.

Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D., Holt, R.D., Hudson, P., Jolles, A., Jones, K.E., Mitchell, C.E., Myers, S.S., Bogich, T. & Ostfeld, R.S. (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, **468**, 647–52.

Knowles, S.C.L., Fenton, A., Petchey, O.L., Jones, T.R., Barber, R. & Pedersen, A.B. (2013) Stability of within-host-parasite communities in a wild mammal system. *Proceedings. Biological sciences*, **280**, 20130598.

Koskela, K.A., Kalin-Mänttäri, L., Hemmilä, H., Smura, T., Kinnunen, P.M., Niemimaa, J., Henttonen, H. & Nikkari, S. (2016) Metagenomic Evaluation of Bacteria from Voles. *Vector-Borne and Zoonotic Diseases*, **17**, 123–133.

Kosoy, M., Mandel, E., Green, D., Marston, E., Jones, D. & Childs, J. (2004) Prospective Studies of Bartonella of Rodents. Part II. Diverse Infections in a Single Rodent Community. *Vector-Borne and Zoonotic Diseases*, **4**, 296–305.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, **79**, 5112–5120.

Krasnov, B.R., Mouillot, D., Shenbrot, G.I., Khokhlova, I.S. & Poulin, R. (2010) Deconstructing spatial patterns in species composition of ectoparasite communities: the relative contribution of host composition, environmental variables and geography. *Global Ecology and Biogeography*, **19**, 515–526.

Kruse, H., Kirkemo, A.M. & Handeland, K. (2004) Wildlife as source of zoonotic infections. *Emerging Infectious Diseases*, **10**, 2067–2072.

Kumar, N., Sharma, S., Barua, S., Tripathi, B.N. & Rouse, T. (2018) Virological and Immunological Outcomes of Coinfections. *Clinical Microbiology Reviews*, **31**, e00111-17.

1320 Lello, J., Boag, B., Fenton, A., Stevenson, I.R. & Hudson, P.J. (2004) Competition and mutualism
1321 among the gut helminths of a mammalian host. *Nature*, **428**, 840–844.
1322 Lello, J., McClure, S.J., Tyrrell, K. & Viney, M.E. (2018) Predicting the effects of parasite co-
1323 infection across species boundaries. *Proceedings. Biological sciences*, **285**, 20172610.
1324 Lindquist, L. & Vapalahti, O. (2008) Tick-borne encephalitis. *The Lancet*, **371**, 1861–1871.
1325 Luis, A.D., Hayman, D.T.S., O'Shea, T.J., Cryan, P.M., Gilbert, A.T., Pulliam, J.R.C., Mills, J.N.,
1326 Timonin, M.E., Willis, C.K.R., Cunningham, A.A., Fooks, A.R., Rupprecht, C.E., Wood,
1327 J.L.N. & Webb, C.T. (2013) A comparison of bats and rodents as reservoirs of zoonotic
1328 viruses: are bats special? *Proceedings. Biological sciences*, **280**, 20122753.
1329 Matar, G.M., Koehler, J.E., Malcolm, G., Lambert-Fair, M.A., Tappero, J., Hunter, S.B. &
1330 Swaminathan, B. (1999) Identification of *Bartonella* species directly in clinical specimens by
1331 PCR-restriction fragment length polymorphism analysis of a 16S rRNA gene fragment.
1332 *Journal of Clinical Microbiology*, **37**, 4045–4047.
1333 Messick, J.B. (2004) Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into
1334 pathogenic potential. *Veterinary clinical pathology / American Society for Veterinary Clinical*
1335 *Pathology*, **33**, 2–13.
1336 Meyer, B.J. & Schmaljohn, C.S. (2000) Persistent hantavirus infections: characteristics and
1337 mechanisms. *Trends in Microbiology*, **8**, 61–67.
1338 Morand, S. (2015) (macro-) Evolutionary ecology of parasite diversity: From determinants of
1339 parasite species richness to host diversification. *International journal for parasitology.*
1340 *Parasites and wildlife*, **4**, 80–87.
1341 Morand, S. & Bordes, F. (2015) Parasite diversity of disease-bearing rodents of Southeast Asia:
1342 habitat determinants and effects on sexual size dimorphism and life-traits. *Frontiers in Ecology*
1343 *and Evolution*, **3**, 110.
1344 Mouillot, D., Šimková, A., Morand, S. & Poulin, R. (2005) Parasite species coexistence and
1345 limiting similarity: a multiscale look at phylogenetic, functional and reproductive distances.

Oecologia, **146**, 269–278.

Moutailler, S., Moro, C.V., Vaumourin, E. & Michelet, L. (2016) Co-infection of Ticks : The Rule Rather Than the Exception. *PLoS Neglected Tropical Diseases*, **10**, e0004539.

Movilla, R., Altet, L., Serrano, L., Tabar, M.D. & Roura, X. (2017) Molecular detection of vector-borne pathogens in blood and splenic samples from dogs with splenic disease. *Parasites and Vectors*, **10**, 1–8.

Murall, C.L., Abbate, J.L., Puelma Touzel, M., Allen-Vercoe, E., Alizon, S., Froissart, R. & McCann, K. (2017) *Invasions of Host-Associated Microbiome Networks*, 1st ed. Elsevier Ltd.

Neimark, H., Johansson, K.E., Rikihisa, Y. & Tully, J.G. (2001) Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of "Candidatus Mycoplasma haemofelis", "Candidatus Mycoplasma haemomuris", "Candidatus Mycoplasma haemosuis" a. *International Journal of Systematic and Evolutionary Microbiology*, **51**, 891–899.

Neimark, H., Mitchelmore, D. & Leach, R.H. (1998) An approach to characterizing uncultivated prokaryotes: the Grey Lung agent and proposal of a *Candidatus* taxon for the organism, "*Candidatus* Mycoplasma ravipulmonis". *International journal of systematic bacteriology*, **48 Pt 2**, 389–94.

Neimark, H., Peters, W., Robinson, B.L. & Stewart, L.B. (2005) Phylogenetic analysis and description of *Eperythrozoon coccoides*, proposal to transfer to the genus *Mycoplasma* as *Mycoplasma coccoides* comb. nov. and request for an opinion. *International Journal of Systematic and Evolutionary Microbiology*, **55**, 1385–1391.

Nunn, C.L., Altizer, S., Jones, K.E. & Sechrest, W. (2003) Comparative Tests of Parasite Species Richness in Primates. *The American Naturalist*, **162**, 597–614.

Pawelczyk, A., Bajer, A., Behnke, J.M., Gilbert, F.S. & Sinski, E. (2004) Factors affecting the component community structure of haemoparasites in common voles (*Microtus arvalis*) from the Mazury Lake District region of Poland. *Parasitology Research*, **92**, 270–284.

1372 Pedersen, A.B., Altizer, S., Poss, M., Cunningham, A.A. & Nunn, C.L. (2005) Patterns of host
1373 specificity and transmission among parasites of wild primates. *International Journal for*
1374 *Parasitology*, **35**, 647–657.
1375 Pedersen, A.B. & Fenton, A. (2007) Emphasizing the ecology in parasite community ecology.
1376 *Trends in Ecology and Evolution*, **22**, 133–139.
1377 Petney, T.N. & Andrews, R.H. (1998) Multiparasite communities in animals and humans:
1378 frequency, structure and pathogenic significance. *International Journal for Parasitology*, **28**,
1379 377–393.
1380 Piasecki, T., Chrzastek, K. & Kasprzykowska, U. (2017) *Mycoplasma pulmonis* of Rodents as a
1381 Possible Human Pathogen. *Vector-Borne and Zoonotic Diseases*, **17**, 475–477.
1382 Pilosof, S., Morand, S., Krasnov, B.R. & Nunn, C.L. (2015) Potential Parasite Transmission in
1383 Multi-Host Networks Based on Parasite Sharing. *PLOS ONE*, **10**, 1–19.
1384 Pitcher, D.G. & Nicholas, R.A.J. (2005) *Mycoplasma* host specificity: Fact or fiction? *Veterinary*
1385 *Journal*, **170**, 300–306.
1386 Poulin, R. (1995) Phylogeny, ecology, and the richness of parasite communities in vertebrates.
1387 *Ecological Monographs*, **65**, 283–302.
1388 Poulin, R., Blanar, C.A., Thieltges, D.W. & Marcogliese, D.J. (2012) Scaling up from
1389 epidemiology to biogeography: local infection patterns predict geographical distribution in fish
1390 parasites. *Journal of Biogeography*, **39**, 1157–1166.
1391 Poulin, R. & Morand, S. (2000) The Diversity of Parasites. *The Quarterly Review of Biology*, **75**,
1392 277–293.
1393 Quiñones-Parra, S.M., Clemens, E.B., Wang, Z., Croom, H.A., Kedzierski, L., McVernon, J.,
1394 Vijaykrishna, D. & Kedzierska, K. (2016) A Role of Influenza Virus Exposure History in
1395 Determining Pandemic Susceptibility and CD8 + T Cell Responses . *Journal of Virology*, **90**,
1396 6936–6947.
1397 R Core Team. (2015) R: A language and environment for statistical computing.

1398 Razzauti, M., Galan, M., Bernard, M., Maman, S., Klopp, C., Charbonnel, N., Vayssier-Taussat,
1399 M., Eloit, M. & Cosson, J.-F. (2015) A Comparison between Transcriptome Sequencing and
1400 16S Metagenomics for Detection of Bacterial Pathogens in Wildlife. *PLOS Neglected Tropical*
1401 *Diseases*, **9**, 1–21.
1402 Rosshart, S.P., Vassallo, B.G., Angeletti, D., Hutchinson, D.S., Morgan, A.P., Takeda, K.,
1403 Hickman, H.D., McCulloch, J.A., Badger, J.H., Ajami, N.J., Trinchieri, G., Pardo-Manuel de
1404 Villena, F., Yewdell, J.W. & Rehermann, B. (2017) Wild Mouse Gut Microbiota Promotes
1405 Host Fitness and Improves Disease Resistance. *Cell*, **171**, 1015-1028.e13.
1406 Sallinen, S., Norberg, A., Susi, H. & Laine, A.L. (2020) Intraspecific host variation plays a key role
1407 in virus community assembly. *Nature Communications*, **11**, 1–11.
1408 Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P.,
1409 Parkhill, J., Loman, N.J. & Walker, A.W. (2014) Reagent and laboratory contamination can
1410 critically impact sequence-based microbiome analyses. *BMC Biology*, **12**, 87.
1411 Salvador, A.R., Guivier, E., Xuéreb, A., Chaval, Y., Cadet, P., Poulle, M.-L., Sironen, T.,
1412 Voutilainen, L., Henttonen, H., Cosson, J.-F. & Charbonnel, N. (2011) Concomitant influence
1413 of helminth infection and landscape on the distribution of Puumala hantavirus in its reservoir,
1414 Myodes glareolus. *BMC Microbiology*, **11**, 30.
1415 Sauvage, F., Penalba, C., Vuillaume, P., Boue, F., Coudrier, D., Pontier, D. & Artois, M. (2002)
1416 Puumala hantavirus infection in humans and in the reservoir host, Ardennes region, France.
1417 *Emerging infectious diseases*, **8**, 1509–1511.
1418 Singer, M. (2010) Pathogen-pathogen interaction: A syndemic model of complex biosocial
1419 processes in disease. *Virulence*, **1**, 10–18.
1420 Smith, K.F., Sax, D.O.V.F. & Lafferty, K.D. (2006) Evidence for the Role of Infectious Disease in
1421 Species Extinction and Endangerment. *Conservation Biology*, **20**, 1349–1357.
1422 Streicker, D.G., Fenton, A. & Pedersen, A.B. (2013) Differential sources of host species
1423 heterogeneity influence the transmission and control of multihost parasites. *Ecology Letters*,

16, 975–984.

Streicker, D.G., Turmelle, A.S., Vonhof, M.J., Kuzmin, I. V, McCracken, G.F. & Rupprecht, C.E. (2010) Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. *Science (New York, N.Y.)*, **329**, 676–9.

Stutz, W.E., Blaustein, A.R., Briggs, C.J., Hoverman, J.T., Rohr, J.R. & Johnson, P.T.J. (2018) Using multi-response models to investigate pathogen coinfections across scales: Insights from emerging diseases of amphibians. *Methods in Ecology and Evolution*, **9**, 1109–1120.

Swanson, S.J., Neitzel, D., Reed, K.D. & Belongia, E.A. (2006) Coinfections acquired from ixodes ticks. *Clinical microbiology reviews*, **19**, 708–727.

Sweeny, A.R., Thomason, C.A., Carbajal, E.A., Hansen, C.B., Graham, A.L. & Pedersen, A.B. (2020) Experimental parasite community perturbation reveals associations between Sin Nombre virus and gastrointestinal nematodes in a rodent reservoir host: SNV-nematode associations in the wild. *Biology Letters*, **16**, 20200604.

Sykes, J.E., Terry, J.C., Lindsay, L.L. & Owens, S.D. (2008) Prevalences of various hemoplasma species among cats in the United States with possible hemoplasmosis. *Journal of the American Veterinary Medical Association*, **232**, 372–379.

Tadin, A., Turk, N., Korva, M., Margaletić, J., Beck, R., Vucelja, M., Habuš, J., Svoboda, P., Zupanc, T.A., Henttonen, H. & Markotić, A. (2012) Multiple co-infections of rodents with hantaviruses, *Leptospira*, and *Babesia* in Croatia. *Vector borne and zoonotic diseases (Larchmont, N.Y.)*, **12**, 388–392.

Tagawa, M., Takeuchi, T., Fujisawa, T., Konno, Y., Yamamoto, S., Matsumoto, K., Yokoyama, N. & Inokuma, H. (2012) A Clinical Case of Severe Anemia in a Sheep Coinfected with *Mycoplasma ovis* and `*Candidatus* Mycoplasma haemovis' in Hokkaido, Japan. *Journal of Veterinary Medical Science*, **74**, 99–102.

Taylor, L.H., Latham, S.M. & Woolhouse, M.E.J. (2001) Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society London B*, **356**, 983–989.

1450 Tegshduuren, E., Yoshimatsu, K., Taruishi, M., Endo, R., Shimizu, K., Koma, T., Yasuda, S.P.,
1451 Kariwa, H., Arikawa, J. & Ishihara, C. (2010) Different cross-reactivity of human and rodent
1452 sera to Tula virus and Puumala virus. *Comparative Immunology, Microbiology and Infectious*
1453 *Diseases*, **33**, e67–e73.
1454 Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S. & Begon, M. (2010)
1455 Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population.
1456 *Science*, **330**, 243–246.
1457 Thumbi, S.M., De. C. Bronsvoort, B.M., Poole, E.J., Kiara, H., Toye, P., Ndila, M., Conradie, I.,
1458 Jennings, A., Handel, I.G., Coetzer, J.A.W., Hanotte, O. & Woolhouse, M.E.J. (2013) Parasite
1459 co-infections show synergistic and antagonistic interactions on growth performance of East
1460 African zebu cattle under one year. *Parasitology*, **140**, 1789–1798.
1461 Vaheri, A., Strandin, T., Hepojoki, J., Sironen, T., Henttonen, H., Mäkelä, S. & Mustonen, J. (2013)
1462 Uncovering the mysteries of hantavirus infections. *Nature Reviews Microbiology*, **11**, 539–
1463 550.
1464 Vaumourin, E., Vourc'h, G., Telfer, S., Lambin, X., Salih, D., Seitzer, U., Morand, S., Charbonnel,
1465 N., Vayssier-Taussat, M. & Gasqui, P. (2014) To be or not to be associated: power study of
1466 four statistical modeling approaches to identify parasite associations in cross-sectional studies.
1467 *Frontiers in Cellular and Infection Microbiology*, **4**, 1–11.
1468 Vayssier-Taussat, M., Moutailler, S., Femenia, F., Raymond, P., Croce, O., La Scola, B., Fournier,
1469 P.E. & Raoult, D. (2016) Identification of novel zoonotic activity of *Bartonella* spp., France.
1470 *Emerging Infectious Diseases*, **22**, 457–462.
1471 Vayssier-Taussat, M., Le Rhun, D., Buffet, J.-P., Maaoui, N., Galan, M., Guivier, E., Charbonnel,
1472 N. & Cosson, J.-F. (2012) *Candidatus* Neoehrlichia mikurensis in bank voles, France.
1473 *Emerging infectious diseases*, **18**, 2063–2065.
1474 Villette, P., Afonso, E., Couval, G., Levret, A., Galan, M., Tatard, C., Cosson, J.F. & Giradoux, P.
1475 (2017) Consequences of organ choice in describing bacterial pathogen assemblages in a rodent

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

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

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

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

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

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

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