Infection, Genetics and Evolution 52 (2017) 10–18

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Liver microbiome of *Peromyscus leucopus*, a key reservoir host species for emerging infectious diseases in North America

A. André [a,b,*], A. Mouton [a], V. Millien [b], J. Michaux [a,c]

[a] Conservation Genetics Laboratory, University of Liège, Boulevard du Rectorat 26, 4000 Liège, Belgium
[b] Redpath Museum, McGill University, 859 Sherbrooke West, Montreal, QC H3A OC4, Canada
[c] CIRAD, UR AGIRs, F-34398 Montpellier, France

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 31 March 2017
Accepted 11 April 2017
Available online 13 April 2017

Keywords:
16S
NGS
Peromyscus leucopus
Microbiome
Borrelia
Bartonella

ABSTRACT

Microbiome studies generally focus on the gut microbiome, which is composed of a large proportion of commensal bacteria. Here we propose a first analysis of the liver microbiome using next generation sequencing as a tool to detect potentially pathogenic strains. We used *Peromyscus leucopus*, the main reservoir host species of Lyme disease in eastern North America, as a model and sequenced V5-V6 regions of the 16S gene from 18 populations in southern Quebec (Canada). The *Lactobacillus* genus was found to dominate the liver microbiome. We also detected a large proportion of individuals infected by *Bartonella vinsonii arupensis*, a human pathogenic bacteria responsible for endocarditis, as well as *Borrelia burgdorferi*, the pathogen responsible for Lyme disease in North America. We then compared the microbiomes among two *P. leucopus* genetic clusters occurring on either side of the St. Lawrence River, and did not detect any effect of the host genotype on their liver microbiome assemblage. Finally, we report, for the first time, the presence of *B. burgdorferi* in a small mammal host from the northern side of the St. Lawrence River, in support of models that have predicted the northern spread of Lyme disease in Canada.

1. Introduction

As many as 6 out of 10 emerging infectious diseases worldwide are zoonotic, which means they are spread by wildlife (Johnson et al., 2015). Infectious diseases cause 9.6 million human deaths yearly (Lozano et al., 2013), while zoonoses represent over 1 billion cases of human illness (Karesh et al., 2012). Salmonellosis, avian flu, Lyme disease, West Nile virus, Dengue, malaria or chikungunya are among the most common zoonotic diseases. The latter three are endemic to the tropics, while the others can also be contracted in temperate regions.

With the recent development of next generation sequencing (NGS) techniques, microbiome-based studies have become increasingly common, allowing direct detection of bacteria without the need to go through any culture steps (Weinstock, 2012). An overwhelming majority of microbiome studies have focused on the gut microbiome, while other organs such as lung, liver or spleen are underrepresented (Can et al., 2014; Yun et al., 2014; Dickson and Huffnagle, 2015). Furthermore, only a few studies have been conducted on wild species' microbiomes (Phillips et al., 2012; Linnenbrink et al., 2013; Minard et al., 2015; Sanders et al., 2015). Yet NGS techniques represent a very promising tool for monitoring the emergence and spread of zoonotic diseases through the direct detection of pathogens in vectors and reservoirs.

Here we screened the microbiome of a common disease reservoir, the white-footed mouse (*Peromyscus leucopus*), a key species in the epidemiology of Lyme disease in eastern North America (Ostfeld et al., 2014). The white-footed mouse is a rodent host for the black-legged tick (*Ixodes scapularis*), the vector of *Borrelia burgdorferi*, the pathogen causing Lyme disease in North America. The white-footed mouse is a highly competent reservoir for *B. burgdorferi*, and can transmit *B. burgdorferi* back to ticks feeding on it in >90% of cases (Mather et al., 1989). *Peromyscus leucopus* is also a reservoir for other human pathogens such as *Bartonella* sp. and *Babesia* sp. (Hofmeister et al., 1998). The *Bartonella* genus in particular was found to be highly prevalent in *P. leucopus* individuals from Minnesota and Wisconsin (Hofmeister et al., 1998).

The white-footed mouse is a widespread rodent species that is found throughout most of eastern North America (Lackey et al., 1985), and the northern limit of its distribution range is shifting rapidly into southern Quebec at an estimated rate of 10 km/yr in response to global warming (Roy-Dufresne et al., 2013). This rapid range expansion is a public health concern in southern Canada as it drives the emergence of new pathogens into naïve regions, including that which causes Lyme disease (Simon et al., 2014). Human cases of Lyme disease have increased over the last decade in Canada, i.e. up to 707 in 2015. In Quebec, the first endemic human case was detected in 2008 and the number of yearly

* Corresponding author at: Conservation Genetics Laboratory, University of Liège, Boulevard du Rectorat 26, 4000 Liège, Belgium.
E-mail address: adrien.andre@ulg.ac.be (A. André).

http://dx.doi.org/10.1016/j.meegid.2017.04.011
1567-1348/

reported cases rose to 160 in 2015 (Gaulin et al., 2016). Monitoring of the bacterial pathogens via *P. leucopus* can thus provide public health agencies in the region with critical information not only for known emerging diseases such as Lyme disease, but also potentially for other emerging pathogens that may not have been detected by disease surveillance programs.

The evolutionary history of a host can be an important driver of their pathogens' dynamics, and distinct host lineages may carry different strains of pathogens that have co-evolved with their respective hosts. A number of recent studies have highlighted the existence of two well differentiated lineages of *P. leucopus* occurring on both sides of the St. Lawrence River in Quebec. These two lineages are distinct in their genetic background (Fiset et al., 2015; Leo et al. in revision; André et al. submitted), but also in their skull morphology (Ledevin and Millien, 2013). Given the significant differentiation of *P. leucopus* from either side of the St. Lawrence River, these populations likely represent two distinct lineages that split several million years ago (Fiset et al., 2015). Both lineages are currently increasing in abundance in southern Quebec and their distribution is shifting along a north-east axis (Roy-Dufresne et al., 2013).

Evidence of the differentiation of bacterial communities across distinct host lineages has been reported for the gut microbiome in humans (Goodrich et al., 2014), apes (Ochman et al., 2010) and bats (Phillips et al., 2012). The gut microbiome is, however, largely composed of commensal bacteria (Goodrich et al., 2014), and consequently may not be the best organ for studying pathogenic strains. Moreover, the gut microbiome is influenced by its host's diet (Turnbaugh et al., 2009), thus reducing the potential effects of the host genotype on the bacterial assemblages. Here we therefore focused on the liver microbiome in *P. leucopus*, an organ without any direct contact with the outer environment nor with allochthonous material. Finally, since the mechanism of *B. burgdorferi* infection on its reservoir host is very complex (Ebady et al., 2016; Gulia-Nuss et al., 2016), we also focused our analyses on lung and spleen samples from *P. leucopus* individuals that had tested positive in PCR screening of heart tissues (Leo et al. in revision), as well as from *P. leucopus* individuals in which we had detected *Borrelia* sp. in the liver using NGS.

This is the first study to characterize bacterial communities inhabiting the liver ecosystem of *P. leucopus* using next generation sequencing. Besides the detection of a number of pathogens of public health interest, we identified *Bartonella* samples to the species level and tested the hypothesis that different *Bartonella* species are associated with distinct *Borrelia* strains, as previously reported (Hofmeister et al., 1998). Active pathogen surveillance studies in Canada are based on PCR analysis of heart tissue samples from small mammal hosts (e.g. Simon et al., 2014). Here we also screened other tissues that might be equally or more susceptible to infection, such as lung and spleen tissues from known infected *P. leucopus* individuals. Finally, we tested the hypothesis that the two distinct host lineages of *P. leucopus* in our study area carried different *B. burgdorferi* strains that could in turn have different levels of pathogenicity. The findings of our study should thus help in refining infectious disease surveillance and management programs by better characterizing the current human pathogen landscape across southern Quebec.

2. Material and methods

2.1. Field sampling

White-footed mice were trapped at 18 field sites across the Monteregie and Estrie regions in southern Quebec (Fig. 1): N1–N3 (northern side of the St. Lawrence River, northern genetic cluster); OR1–OR5 (western side of the Richelieu River) and ER (eastern side of the Richelieu River), both of which characterized the southern genetic cluster (Fiset et al., 2015; André et al. submitted). At each site, 160 Sherman traps were placed every 10 m in grids of 40 traps during the summer of 2013 and 2014. The traps were baited with a mixture of oats and peanut butter in the late afternoon and checked the following morning for three consecutive nights. Captured animals were euthanized with an overdose of isofluorane, followed by cervical dislocation. *Peromyscus* individuals were identified to the species level using species-specific primers, as described in Rogic et al. (2013), to distinguish them from *P. maniculatus* individuals. All procedures were approved by the Ministère des Ressources Naturelles et de la Faune du Québec (SEG permits #2013-07-04-14-16-17-SF and #2014-05-02-1638-05-16-SF), and the McGill University Animal Care Committee (AUP#5420).

2.2. Genetic analysis

2.2.1. DNA extraction

Total DNA was extracted from liver ($N = 203$, Table 1), lung ($N = 12$) and spleen ($N = 12$) tissue samples using the DNeasy™ Tissue Kit (Qiagen®, Hilden) according to a protocol designed for bacterial DNA harvesting. The protocol is described in detail in the Supporting Information.

2.2.2. 16s gene sequencing

We amplified a ~142 bp fragment of the 16S rRNA variable regions 5 and 6 (V5–V6) using the 784F-1061R primer pair originally designed by Andersson et al. (2008), coupled with Illumina overhand adapters. One negative extraction control was added to every batch of 24 samples, and two additional negative controls were added for the PCR steps. A mock community sample (HM-783D, BEI resources) containing genomic DNA from 20 bacterial strains, at concentrations ranging from 0.6 to 1400 pg/μl, was also added in triplicate to confirm the reliability of our method. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter Life Sciences, IN, USA). Purified amplicons were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo Scientific, MA, USA) according to the manufacturer's protocol on a fluorimeter (FilterMax F3, Molecular Devices). Quantified products were then pooled at equimolarity and sent to the GIGA Genomics platform (ULg) for sequencing on an ILLUMINA MiSeq V2 benchtop sequencer. The complete protocol is described in the Supporting Information.

2.3. Bioinformatics

Raw sequences were processed using a script consisting of a mix of FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit; 23-09-16) and USEARCH (Edgar, 2010) functions. Briefly, the paired-end reads were joined on their overlapping ends. The overlap had to be at least 10 bases long with an 8% maximum difference. Primers were removed and sequences were filtered to keep only those with at least 90% of the bases with a quality index greater than Q30. Chimeric sequences were detected and discarded using the uchime_ref function (Edgar et al., 2011) combined with the chimera.slayer reference database from the Broad Microbiome Utilities (Haas et al., 2011). Operational taxonomic unit (OUT) clustering was performed using the cluster_otus function from usearch package (Edgar, 2010), with an OTU radius of 3%. As pointed out by Salter et al. (2014), some sequences may be the result of contaminants present in the extraction or PCR reagents. OTUs were therefore not included in subsequent analyses when their relative abundance was not at least 10-fold greater than in any of the negative controls, according to Minard et al. (2015). This filter is quite restrictive and might silence a few OTUs actually present in some samples, but it has the advantage of silencing false-positive taxa. The remaining OTUs were assigned to their corresponding genus using the RDP-classifier tool (version 2.10.1) (Wang et al., 2007), with an assignment confidence cutoff of 0.8.

As the number of resulting sequences per sample ranged from 12 to 111,572, we standardized the number of sequences per sample to account for the effects of uneven sampling on the community metrics.

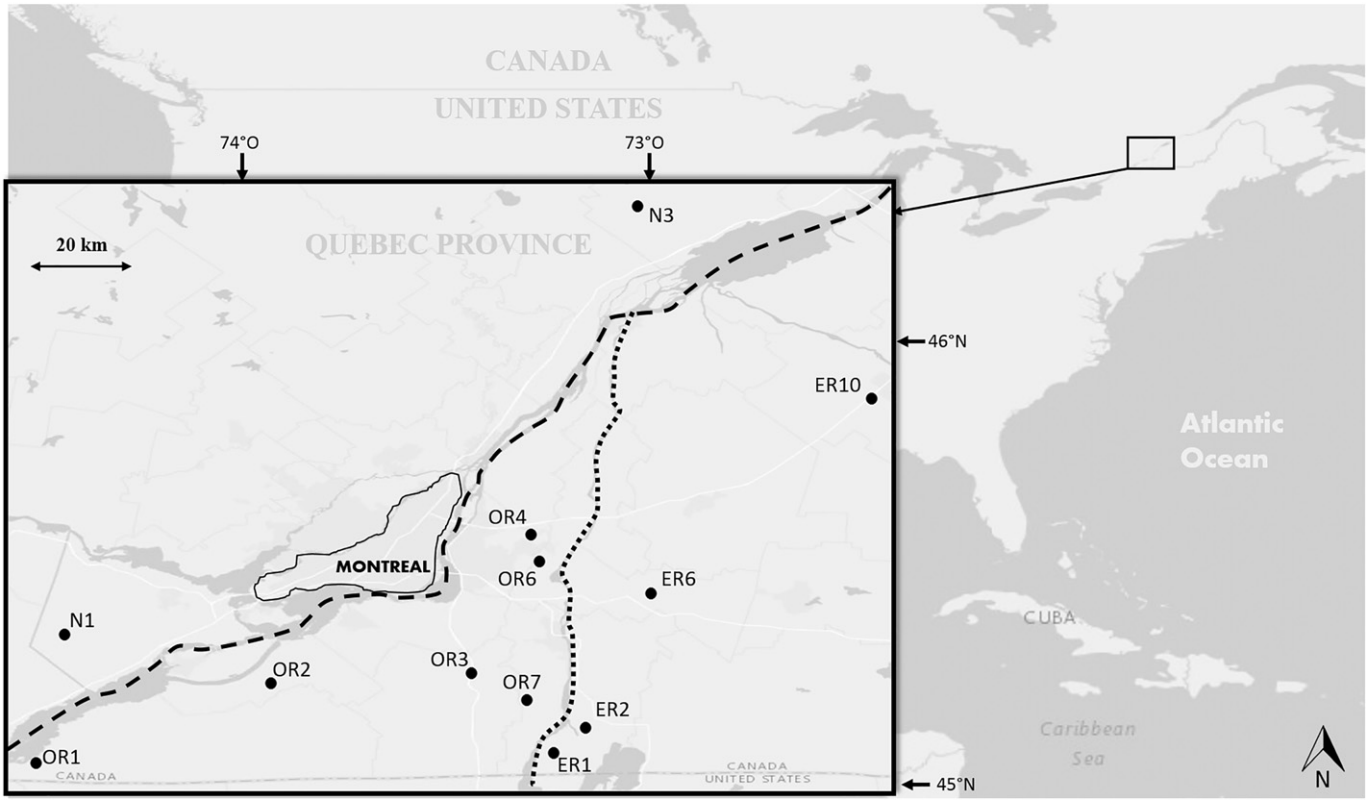

Fig. 1. Distribution of the sampling sites. (N1, N3: northern genetic cluster; OR1, OR2, OR3, OR4, OR6, OR7, ER2, ER6, ER10: southern genetic cluster). The Saint-Lawrence and Richelieu rivers are represented by the dashed and dotted lines respectively.

Samples with a number of reads above a threshold value of 1000 were subsampled to this threshold value using the rrarefy function from the VEGAN (Oksanen et al., 2013) R package (R Core Team, 2016).

Samples from individuals that tested positive for *B. burgdorferi* in a PCR analysis on heart tissues (Leo et al., in revision), or presenting more than two sequences attributed to *Borrelia* sp. in our first analysis, were selected for a second analysis. This time, liver, lung and spleen tissues were processed for these individuals according to the protocol detailed above.

2.4. Bartonella species identification

Twenty DNA extracts identified as carrying *Bartonella* strains were used as starting material for species identification according to the protocol developed by Maggi and Breitschwerdt (2005). Briefly, a PCR was performed using 321s (5′ AGA TGA TGA TCC CAA GCC TTC TGG 3′) and 983as (5′ TGT TCT YAC AAC AAT GAT GAT G 3′) oligonucleotides as sense and antisense primers. This primer pair was designed to distinguish the different species from the genus according to their amplicon sizes. PCR products were visualized on a polyacrylamide gel and sent to Eurofins Genomics (Ebensburg, Germany) for Sanger sequencing.

Table 1
Sample size (*N*) and occurrence of *Borrelia* sp. and *Bartonella* sp. within each sampling site.

Site	*N*	Occurrence	
		Borrelia	*Bartonella*
N1	18	0	10
N3	21	1	7
ER1	20	1	9
ER2	10	1	2
ER6	8	0	2
ER10	20	4	6
OR1	23	5	10
OR2	35	1	23
OR3	19	0	7
OR4	24	5	6
OR6	2	1	0
OR7	3	1	1
Total	203	20	83

2.5. Statistical analysis

We assessed the dissimilarity between liver microbiomes across individuals using four distinct metrics. The Bray-Curtis dissimilarity index is based on weighting taxon abundance, while the Jaccard distance relies on presence/absence only. The weighted and unweighted UniFrac indices are also based on weighting taxon abundance and presence/absence, respectively, but they incorporate the phylogenetic distances between bacterial communities by comparing the unique vs. shared branch lengths underlying these communities (Lozupone and Knight, 2005). According to Linnenbrink et al. (2013), permutational multivariate analysis of variance was then performed on these four dissimilarity matrices using the Adonis function from the VEGAN R package (Oksanen et al., 2013) to detect significant differences in the microbiomes between the two *P. leucopus* genetic lineages (Fiset et al., 2015; André et al., submitted).

A heatmap was drawn up to plot the rarefied abundances from the 60 most prevalent genera in each sample using the plot_heatmap function (Rajaram and Oono, 2010) in the phyloseq package (McMurdie and Holmes, 2013) on R (R Core Team, 2016). We also produced a barplot representing the abundances and prevalences of the most common genera using the barplot function. We then further explored the *P. leucopus* infection pattern with two specific emerging pathogens, i.e. *Borrelia* sp. and *Bartonella* sp. The association between *Borrelia* sp. and *Bartonella* sp. occurrences was tested using Kendall's correlation test. To determine the most suitable organ for *Borrelia* sp. detection, a Wilcoxon signed-rank test was performed to highlight any significant

difference in the number of *Borrelia* sequences detected among the lung, liver and spleen tissues sampled. We then assessed the effects of the infection intensity (number of *Bartonella* sp. sequences per infected mouse) on the infection prevalence (proportion of mice infected by *Bartonella* sp.) at each sampling site with a linear model using the lm function in R. Two sampling sites with less than eight individuals were not included in this latter analysis. Due to small sample size, this last analysis was not completed for *Borrelia* sp.

3. Results

Three replicates of mock community samples containing known concentrations of genomic DNA from 20 bacterial strains were sequenced. All of them gave consistent results (Table S1): 17 of the 20 different strains originally included in the sample were detected. The three undetected strains were present at the lowest concentrations. Therefore, our protocol allowed bacterial DNA detection and identification to the genus level as long as its concentration in the DNA extract was at least 3.2 pg/μl, and provided that the sequence was included in the reference database.

3.1. Liver microbiome

Following the raw data processing, we obtained 5,003,515 sequences belonging to 22,854 distinct OTUs. Using our negative controls, we discarded 3775 contaminant OTUs, thus leaving 1,665,384 uncontaminated sequences. The rate of discarded contaminant sequences reached 66.7% of the processed sequences. Among the uncontaminated sequences, 1,098,461 were successfully attributed to the genus level and used for subsequent analyses. Table S2 (Supporting Information) presents the number of sequences for each sample after each main processing step. The number of sequences per sample ranged from 12 to 111,572. Although a correlation was noted between the sequence initial input and final output (cor = 0.6), the uneven sequence output was mainly based on the contaminant read rate. A highly significant correlation (0.83) was found between the sample total processing rate and the contaminant sequence rate. Because all samples were carefully processed according to the same laboratory protocol in strictly similar conditions, these disparities in the contaminant sequence rate were more likely due to low bacterial DNA concentrations within the liver rather than high contaminating bacterial DNA concentrations. This disparity between liver bacterial DNA concentrations might therefore have been the result of high bacterial infection in some liver samples and relatively low rates in others. Further research would nevertheless be required to confirm this hypothesis.

The rarefied dataset returned 469 bacterial genera, 13 of which presented a read number >1% of the total rarefied abundance. Overall, no clear distinction between the two *P. leucopus* lineages was apparent based on their microbiome (Fig. 2). The most abundant genus in our samples was *Lactobacillus* (accounting for 22.3% of the total number of reads), followed by *Bartonella* (19%), *Staphylococcus* (7%) and *Gemella* (6.2%) (Fig. 2). All other genera accounted for <2% of the total number of reads (Fig. 3). The most prevalent genera were *Staphylococcus* and *Lactobacillus*, both present in 66% of the samples. 60 other genera were present in >10% of the samples (Fig. 3).

We further explored the *Bartonella* sp. and *Borrelia* sp. infection patterns in our liver samples (Table 1). The proportion of *Bartonella*-positive mice within each sampling site increased with the number of *Bartonella* reads per positive mouse, regardless of whether we considered the mean ($F = 22.1$ $p = 0.0015$, $r^2 = 0.73$, Fig. 4) or median ($F = 11.06$, $p = 0.01044$, $r^2 = 0.58$, Fig. 4) intensities. No association was found between *Bartonella* and *Borrelia* occurrences (Kendall's tau $= -0.049$, $p = 0.49$). We then used a specific primer pair for *Bartonella* on 20 randomly selected *Bartonella*-positive samples. Each of the tested samples showed the same amplicon size on polyacrylamide gel (data not shown), revealing the presence of a single species (Maggi and Breitschwerdt, 2005). Sanger sequencing was performed in duplicate and the 381 bp sequences obtained matched the deposited *Bartonella vinsonii arupensis* subspecies by 98%.

3.2. Effect of host phylogeny on the liver microbiome

Three of the four computed distances (Table 2) (Jaccard, Bray-Curtis and weighted UniFrac) did not show any significant difference between the two distinct genetic clusters among the bacterial communities. The unweighted UniFrac index did return a significant difference, but with a very small r^2 value (Table 2).

3.3. Borrelia burgdorferi prevalence across different organs

Analyses performed at the Public Health Agency of Canada on heart tissue samples from our specimens and using a nested PCR protocol targeting two *Borrelia*-specific markers (e.g. Ogden et al., 2011) led to the detection of six mice infected by *B. burgdorferi* (Table 3). Our analysis of liver tissues confirmed the detection of *Borrelia* sp. in four out of these six individuals and enabled detection of an additional 14 infected mice, including two mice that returned positive results only before the rarefaction step. A complementary analysis was then conducted on mice previously detected as *Borrelia*-positive by PCR and on those presenting more than two sequences attributed to *Borrelia* sp. in the present study, but here DNA extracted from lung and spleen tissues was used. Out of the three tested organs, the lungs appeared to be the best choice for detecting *Borrelia* sp. Their sequencing led to the detection of 11 of the 12 positive samples tested with, the highest number of *Borrelia* sp. sequences detected, as compared to the liver (Wilcoxon signed-rank test, $p < 0.042$) and spleen (Wilcoxon signed-rank test, $p < 0.007$) finding. The liver and spleen sequencing led to the detection of only 10 and 5 infected mice, respectively, out of the 12 analysed, with no significant difference in the number of *Borrelia* sp. sequences detected between the two organs (Wilcoxon signed-rank test, $p = 0.056$).

4. Discussion

Our main objective was to characterize the liver microbial community in *P. leucopus* individuals. We further explored infection patterns in *Bartonella* and *Borrelia* genera and examined the effect of the host phylogeny on the liver microbiome.

Our study highlighted the importance of including negative controls in NGS protocols, as 66.7% of the total number of processed reads were diagnosed as contaminant and were therefore discarded. The dominance of contaminant reads in our study confirmed the relatively low microbial biomass present in liver tissues (Salter et al., 2014). To avoid drawing erroneous conclusions, we therefore recommend systematic inclusion of several negative control samples in study designs involving low microbial biomass samples. The mock community sample processing results confirmed the reliability of our protocol, and we were able to detect DNA of all bacterial strains originally present in our samples at above 3.2 pg/μl concentration. Furthermore, once the contaminants were discarded, no additional DNA was detected in our samples, thereby validating our protocol.

We found that *Lactobacillus* sp. dominated the liver microbiome of *P. leucopus*. These bacteria were the most abundant genus among all samples (22.3% of the total number of reads), followed by *Bartonella* (19%), *Staphylococcus* (7%) and *Gemella* (6.2%). All other genera were represented by <2% of the total number of reads. In addition to being the most abundant genus detected within the samples, *Lactobacillus* was also the most prevalent genus between samples, with 60% of all mouse liver tissues found to be *Lactobacillus*-positive.

Lactobacillus is a genus of gram-positive facultative anaerobic bacteria belonging to the Firmicutes phylum. It was reported to be the third most abundant genus in both *P. leucopus* and *P. maniculatus gracilis* gut microbiota (Baxter et al., 2015), while also being the most abundant

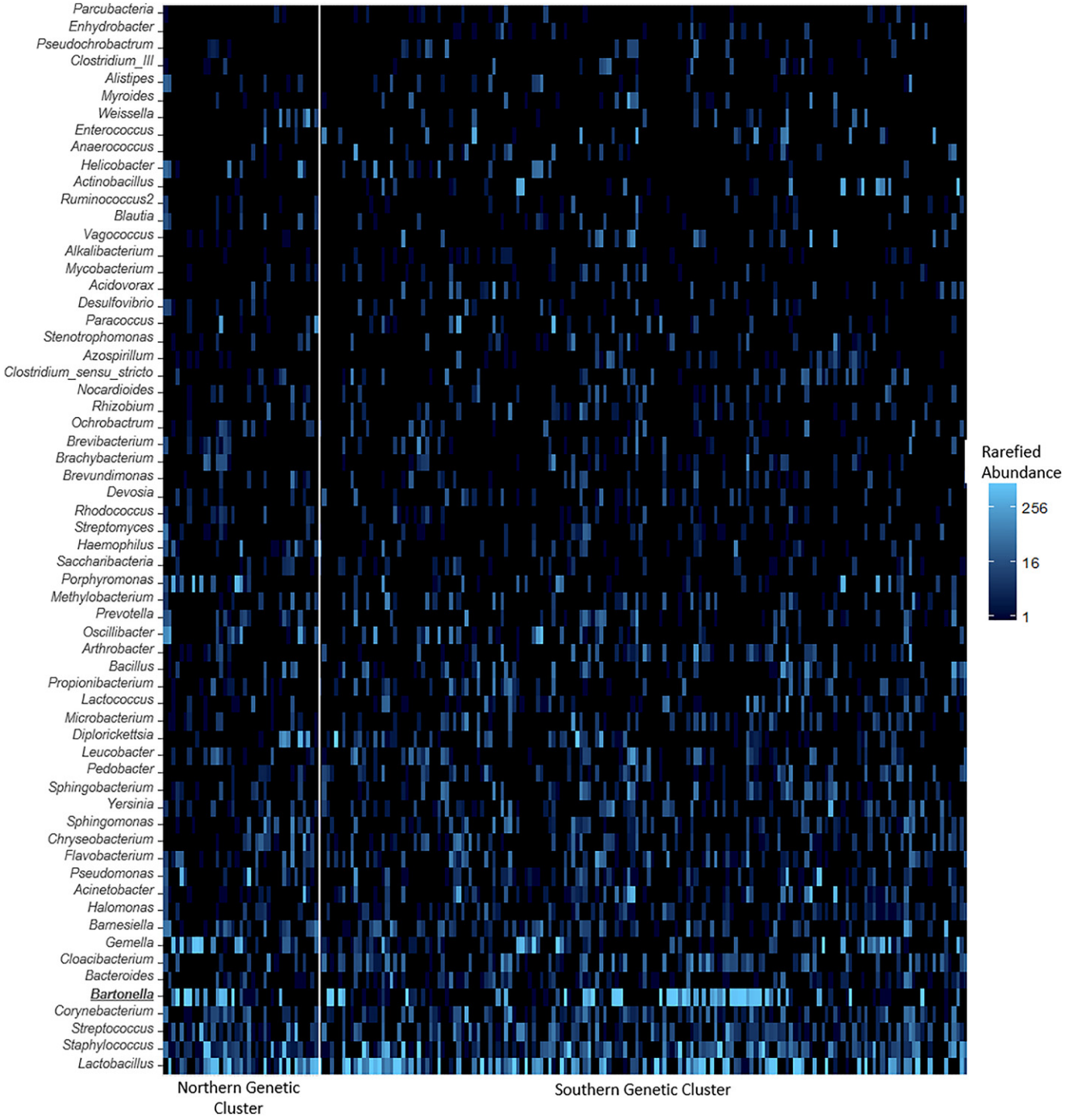

Fig. 2. Heatmap representing the rarefied abundances from the most abundant genera in each sample. Genera are arranged in order of increasing prevalence from the top to the bottom. Samples on the x axis are ordered according to the genetic cluster they belong to.

bacterium genus in human vaginal microbiota (Nardis et al., 2013). Little is known about its role in the gut microbiota. Walter (2008) suggested that only a small number of *Lactobacillus* species are true inhabitants of the mammalian intestinal tract and argued that these bacteria are mostly allochthonous members derived from fermented food. *Lactobacillus* species have a clearer role in human vaginal microbiota, i.e. protecting the vaginal ecosystem by competing with other microorganisms for nutrients and adherence to the vaginal epithelium, lowering the vaginal PH, producing lactic acid and other organic acids, producing antimicrobial substances, and modulating the local immune system (Aroutcheva et al., 2001). Since the liver has no direct contact with the outside environment, its *Lactobacillus* community is less likely to be allochthonous and may play a similar protection role.

Bartonella was the second most abundant genus found among all of our liver samples. This genus contains >23 different species (Edouard et al., 2015), at least 11 of which are known to be pathogenic for humans, with infections producing a wide range of symptoms, especially endocarditis and high fever (Breitschwerdt and Kordick, 2000). It is transmitted by blood-sucking arthropods, such as ticks or fleas, and the reservoir hosts are generally mammal species. Here, Sanger sequencing revealed 98% identity with the deposited *B. vinsonii arupensis* subspecies, which is known to be pathogenic for humans, with a few cases of infection

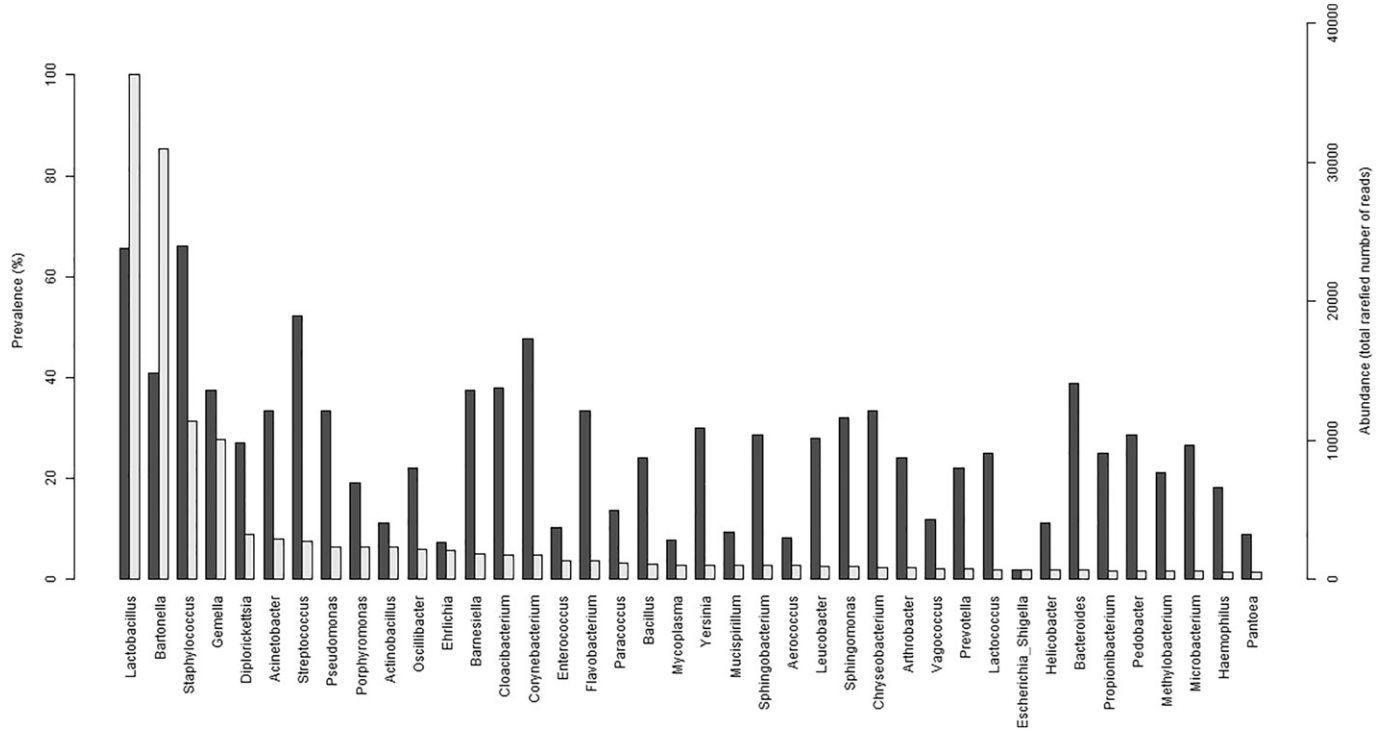

Fig. 3. Prevalence (dark grey bars; left axis) and abundance (light grey bars; right axis), of the genera with a total abundance >2% of the total number of rarefied reads.

reported in Wyoming (Welch et al., 1999), Europe (Fenollar et al., 2005; Melzi et al., 2015) and Thailand (Bai et al., 2012). Reported clinical symptoms include fever, chill, fatigue, myalgia, icterus, headache and hepatic pain. This *Bartonella* subspecies has also been observed in 84.2% of 737 captured deer mice (*Peromyscus maniculatus*) in a study performed in Colorado (Bai et al., 2011). This high prevalence was explained by the high persistency of the bacteria, with infection lasting more than a year in some individuals. In our study, the overall prevalence was 40.9%, but varied across sampling sites, with values ranging from 0 to 65.7%. Interestingly, the infection intensity among infected individuals (measured by the rarefied number of *Bartonella* reads) was positively correlated to the proportion of infected mice at the sampling site. Hence, sites with the highest number of infected mice also contained the most infected mice. This was not surprising given that Bartonellosis is a disease mostly transmitted by blood-sucking arthropod vectors (Bai et al., 2011). The latter are more likely to acquire bacteria from highly infected mice during the feeding process, and thus more susceptible to infect other mice, which in turn could also transmit the disease, leading to exponential spreading of the contagious bacteria.

Contrary to the findings of a study focused on *P. leucopus* populations from upper midwestern USA (Hofmeister et al., 1998), we found no associations between *Bartonella* and *Borrelia* infection prevalences. The strict association between a *Bartonella* species closely related to *B. vinsonii*, and *B. burgdorferi* reported by Hofmeister et al. (1998) may therefore have been dependent on the *Bartonella* species involved, and the findings would thus not be applicable to the entire genus.

Active Lyme disease surveillance in Canada involves the analysis of heart tissue samples from small mammal hosts (e.g. Simon et al.,

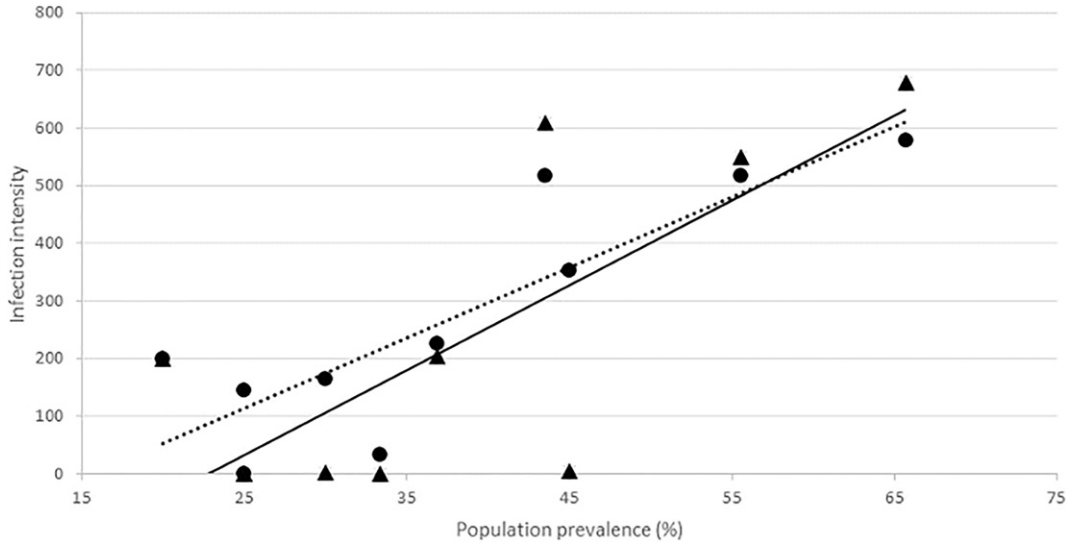

Fig. 4. Relation between the prevalence of *Bartonella* within each population and the mean (circles, dashed line; $y = 12{,}205x - 190.25$; $r^2 = 0.73$) and median (triangles, solid line; $y = 14.698x - 333.46$; $r^2 = 0.58$) *Bartonella* infection intensities within infected mice.

Table 2
Summary of PERMANOVAs performed on the four dissimilarity matrices between the two genetic clades.

Dissimilarity index	*p*-Value	r^2
Bray-Curtis	0.236	0.006
Jaccard	0.275	0.006
Weighted Unifrac	0.550	0.004
Unweighted Unifrac	0.044	0.007

2014), and six of our *P. leucopus* individuals tested positive for *B. burgdorferi* via this analysis. Here, based on liver DNA extracts and using NGS methods, we detected *Borrelia* sp. in only four out of these six individuals. However, we detected 14 additional infected mice (Table 3). The number of *Borrelia* sequences obtained for these latter samples were sometimes very low, which may explain why a PCR-based method would not detect them. The *Borrelia* concentration within a given infected individual could therefore vary across organs, depending on the infection stage or on the differential dissemination pattern, as suggested by Dykhuizen et al. (2008). We thus performed a second analysis on the six individuals that tested positive for *B. burgdorferi* by PCR, as well as six individuals that presented more than two *Borrelia* sequences in our sequencing. We performed this second analysis using DNA extracted from two additional organs, i.e. lung and spleen, which are often used in epidemiology studies (Casswall et al., 2010; Galan et al., 2016). The spleen analysis only confirmed the presence of *Borrelia* in 5 out of the 12 samples. By contrast, our lung analysis confirmed the presence of *Borrelia* in 11 out of the 12 samples. Moreover, for a given individual, there were significantly more *Borrelia* sequences in the lung than in the spleen or the liver, indicating the higher sensitivity of lung tissues.

These results—although based on a small number of samples, sometimes with only a few *Borrelia* sequences per sample—should be considered with caution. However, they do suggest that out of the three tested organs, the lung would be the best candidate for *Borrelia* detection and should therefore be used in future studies until another organ generates better results. We also hypothesize that additional infected samples would have been detected if lung tissues had been used as starting material for the entire sample set.

The high *Borrelia* concentration found in lung tissue may have been due to the migration of bacteria from other neighboring tissues rather than to contamination from the respiratory tract, as such an infection process has yet to be documented (Ostfeld, 2011). However, is not clear why *Borrelia* strains appear to have a physiological affinity for lung tissue.

Table 3
Borrelia sp. detection using PCR targeting two markers for *B. burgdorferi*, and rarefied number of *Borrelia* reads within livers (up to 3 replicates), spleens and lungs using NGS. (*) = number of reads before the rarefaction step but silenced after it. (/) = not tested.

Sample	Classic PCR	Liver1	Liver2	Liver3	Spleen	Lung
1630	Positive	12	0	0	0	952
1667		44	10	0	0	971
1710		0	1	0	0	98
1793		227	49	/	0	403
1954		0	/	/	0	581
1970		165	186	/	25	45
1555	Negative	3	0	/	0	19
1595		37	0	/	72	8
1717		21	0	/	11	80
1719		5	0	/	0	2
1783		28	4	/	1	0
1792		22	19	/	6	477
1615		1	/	/	/	/
1835		1	/	/	/	/
1951		1	/	/	/	/
1957		2	/	/	/	/
1971		1	/	/	/	/
2009		2	/	/	/	/
1455		6*	/	/	/	/
1631		2*	/	/	/	/

Moreover, out of the 20 *Borrelia*-infected mice, one had been captured on the northern side of the St. Lawrence River, which represents the first detection of *Borrelia* in a small mammal host in this area. *Borrelia burgdorferi* was detected in this area in questing ticks (Simon et al., 2014), but these pests were likely from an adventitious population brought into southern Quebec by migratory birds and that it did not acquire the pathogen locally (Leo et al., 2016). By contrast, because of its more limited dispersal ability, the detection of one infected *P. leucopus* individual on the northern side of the St. Lawrence River supports model predictions of the potential northern spread of Lyme disease in Canada, while also indicating that *B. burgdorferi* transmission cycles are becoming endemic in areas previously free of the pathogen (Simon et al., 2014).

Microbiome composition is determined by three factors: microbial immigration, microbial elimination, and the relative reproduction rates of its members (Dickson and Huffnagle, 2015). Microbial immigration may be the result of the direct input of bacteria from the outer environment, through feeding (Lynch et al., 2009), breathing (Gibson et al., 2003), or from insect vector bites (Radolf et al., 2012). It also may be the result of internal migration by active or passive processes between organs (Balzan et al., 2007). Lastly, some bacteria are known to be vertically transmitted (Ley et al., 2005).

In the gut and lungs, i.e. the two most studied organs with regard to their microbiomes in mammals, a large proportion of microbial immigration occurs during the feeding and breathing processes. In comparison, the liver has no direct opening or contact with the outer environment. Its microbiome is thus probably less influenced by the outer environment and would instead be the result of internal bacterial translocation and vertical transmission. Hence, and due to the fact that microbial elimination is a task managed by the host immune system, which is highly dependent on the host genetic makeup, we expected to find a greater effect of the host phylogeny on the liver than on the gut microbiome composition. Two previous studies (Fiset et al., 2015; Andre et al. submitted) based on several mitochondrial DNA markers as well as on the major histocompatibility complex DRB marker, highlighted the presence of two genetically distinct clusters of *P. leucopus* in southern Quebec, separated by the St. Lawrence River. These two genetic clusters have evolved separately for at least 2 Ma (Fiset et al., 2015) and populations from the two clusters are located only few kilometers apart under similar environmental conditions. Contrary to our expectations, we did not detect any major differences in the liver microbiome composition between these populations. Out of the four differentiation measures we used, only one revealed a significant, yet rather low, difference. This apparent lack of an effect of the host genetic structure and evolutionary history on the liver microbiome is consistent with the findings of a study of wild European *M. musculus* populations, in which genetic differentiation explained only 6% of the total variation with regard to the caecal mucosa-associated bacterial community, while no significant influence was observed on the caecal content community (Linnenbrink et al., 2013). Similar results were obtained at a slightly larger scale by Baxter et al. (2015) who reported that intra- and inter-individual variations masked interspecific variation in the gut microbiota of sympatric *Peromyscus* populations. Studies that have detected a marked effect of the host phylogeny were conducted on a much broader scale, comparing gut microbiota across orders (Phillips et al., 2012) and families (hominids) (Ochman et al., 2010). Yet, even at these broad scales, different species sharing a common environment have been found to have convergent gut microbiota (Moeller et al., 2013). Among our *P. leucopus* populations, the lack of effect of genetic structure on their liver microbiome composition is in line with this conclusion, and may be the result of indirect environmental immigration into the liver. The two *P. leucopus* clusters in our study were geographically very close and inhabited a similar environment. Bacterial

strains from the environment might have entered the body of these mice via the digestive or respiratory tract to then migrate to the liver by active or passive translocation pathways. In summary, differentiation between the two *P. leucopus* genetic clusters did not have any significant effect on the liver microbiome and horizontal transmission from the environment may therefore be a stronger factor in shaping liver bacteria communities.

5. Conclusion

In this study, we characterized the liver microbiome from *Peromyscus leucopus* individuals, dominated by the *Lactobacillus* genus. Our NGS method combined with the use of multiple organs appeared to be more effective for the detection of *B. burgdorferi*. We highlighted, for the first time, the presence of the bacteria in a small mammal host from the northern side of the St. Lawrence River, confirming model findings predicting the northern spread of Lyme disease in Canada. We also detected *Bartonella* strains at a high prevalence and identified them as being members of the *B. vinsonii arupensis* subspecies. Finally, we found almost no effect of the host genotype on the liver microbiome between the two genetic clusters of mice separated by at least 2 Ma.

Data accessibility

All fastq files were deposited at EMBL European Nucleotide Archive (https://www.ebi.ac.uk/ena) under the accession number ERP018195.

Acknowledgements

We thank S. Leo, S. Turney, field assistants and land owners. We also thank R. Lindsay (PHAC) for initial *Borrelia* screening. Computational resources were provided by the CBGP HPC computational platform, and by the Consortium des Équipements de Calcul Intensif (CÉCI) funded by the Fonds de la Recherche Scientifique de Belgique (F.R.S.-FNRS) under Grant No. 2.5020.11. The following reagent was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Staggered, Low Concentration), v5.2L, for 16S rRNA Gene Sequencing, HM-783D. This work was supported by Belgian FRS-FNRS (Fonds de la Recherche Scientifique) fellowship to AA and to JRM and by financial grants from the Belgian FRS-FNRS ("credits pour brefs séjours à l'étranger") to AA and JRM, and from the "Patrimoine de l'université de Liège" to AA. VM is supported by a NSERC Discovery Grant (RGPIN/341918-2012).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2017.04.011.

References

Andersson, A.F., Lindberg, M., Jakobsson, H., Bäckhed, F., Nyrén, P., Engstrand, L., 2008. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PloS one 3 (7) e2836.

Aroutcheva, A., Gariti, D., Simon, M., Shott, S., Faro, J., Simoes, J.A., Gurguis, A., Faro, S., 2001. Defense factors of vaginal lactobacilli. Am. J. Obstet. Gynecol. 185, 375–379.

Bai, Y., Calisher, C.H., Kosoy, M.Y., Root, J.J., Doty, J.B., 2011. Persistent infection or successive reinfection of deer mice with *Bartonella vinsonii* subsp. arupensis. Appl. Environ. Microbiol. 77, 1728–1731.

Bai, Y., Kosoy, M.Y., Diaz, M.H., Winchell, J., Baggett, H., Maloney, S.A., Boonmar, S., Bhengsri, S., Sawatwong, P., Peruski, L.F., 2012. *Bartonella vinsonii* subsp. arupensisin Humans, Thailand. Emerg. Infect. Dis. 18, 989–991.

Balzan, S., De Almeida Quadros, C., De Cleva, R., Zilberstein, B., Cecconello, I., 2007. Bacterial translocation: overview of mechanisms and clinical impact. J. Gastroenterol. Hepatol. 22, 464–471.

Baxter, N.T., Wan, J.J., Schubert, A.M., Jenior, M.L., Myers, P., Schloss, P.D., 2015. Intra- and interindividual variations mask interspecies variation in the microbiota of sympatric peromyscus populations. Appl. Environ. Microbiol. 81, 396–404.

Breitschwerdt, E.B., Kordick, D.L., 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin. Microbiol. Rev. 13, 428–438.

Can, I., Javan, G.T., Pozhitkov, A.E., Noble, P.A., 2014. Distinctive thanatomicrobiome signatures found in the blood and internal organs of humans. J. Microbiol. Methods 106, 1–7.

Casswall, T.H., Nemeth, A., Nilsson, I., Wadstrom, T., Nilsson, H.O., 2010. Helicobacter species DNA in liver and gastric tissues in children and adolescents with chronic liver disease. Scand. J. Gastroenterol. 45, 160–167.

Dickson, R.P., Huffnagle, G.B., 2015. The lung microbiome: new principles for respiratory bacteriology in health and disease. PLoS Pathog. 11, e1004923.

Dykhuizen, D.E., Brisson, D., Sandigursky, S., Wormser, G.P., Nowakowski, J., Nadelman, R.B., Schwartz, I., 2008. The propensity of different *Borrelia burgdorferi* sensu stricto genotypes to cause disseminated infections in humans. Am. J. Trop. Med. Hyg. 78 (5), 806–810.

Ebady, R., Niddam, A.F., Boczula, A.E., Kim, Y.R., Gupta, N., Tang, T.T., Moriarty, T.J., 2016. Biomechanics of Borrelia burgdorferi vascular interactions. Cell Reports 16 (10), 2593–2604.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

Edouard, S., Nabet, C., Lepidi, H., Fournier, P.E., Raoult, D., 2015. *Bartonella*, a common cause of endocarditis: a report on 106 cases and review. J. Clin. Microbiol. 53, 824–829.

Fenollar, F., Sire, S., Raoult, D., 2005. *Bartonella vinsonii* subsp. arupensis as an agent of blood culture-negative endocarditis in a human. J. Clin. Microbiol. 43, 945–947.

Fiset, J., Tessier, N., Millien, V., Lapointe, F.J., 2015. Phylogeographic structure of the white-footed mouse and the deer mouse, two Lyme disease reservoir hosts in Quebec. PLoS One 10, e0144112.

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., Bik, H., 2016. 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. mSystems 1 (e00032-00016).

Gaulin, C., Leblanc, M.A., Mercier, M., Valiquette, L., Bédard, D., Markowski, F., Lambert, L., Petit, G., Poirier, B., Picard, I., 2016. Flash vigie. Bulletin québécois de vigie, de surveillance et d'intervention en protection de la santé publique 11.

Gibson, R.L., Burns, J.L., Ramsey, B.W., 2003. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am. J. Respir. Crit. Care Med. 168, 918–951.

Goodrich, Julia K., Waters, Jillian L., Poole, Angela C., Sutter, Jessica L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, Jordana T., Spector, Timothy D., Clark, Andrew G., Ley, Ruth E., 2014. Human genetics shape the gut microbiome. Cell 159, 789–799.

Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Sonenshine, D.E., Roe, R.M., Waterhouse, R.M., Sattelle, D.B., de la Fuente, J., Ribeiro, J.M., Megy, K., Thimmapuram, J., Miller, J.R., Walenz, B.P., Koren, S., Hostetler, J.B., Thiagarajan, M., Joardar, V.S., Hannick, L.I., Bidwell, S., Hammond, M.P., Young, S., Zeng, Q., Abrudan, J.L., Almeida, F.C., Ayllón, N., Bhide, K., Bissinger, B.W., Bonzon-Kulichenko, E., Buckingham, S.D., Caffrey, D.R., Caimano, M.J., Croset, V., Driscoll, T., Gilbert, D., Gillespie, J.J., Giraldo-Calderón, G.I., Grabowski, J.M., Jiang, D., Khalil, S.M.S., Kim, D., Kocan, K.M., Koči, J., Kuhn, R.J., Kurtti, T.J., Lees, K., Lang, E.G., Kennedy, R.C., Kwon, H., Perera, R., Qi, Y., Radolf, J.D., Sakamoto, J.M., Sánchez-Gracia, A., Severo, M.S., Silverman, N., Šimo, L., Tojo, M., Tornador, C., Van Zee, J.P., Vázquez, J., Vieira, F.G., Villar, M., Wespiser, A.R., Yang, Y., Zhu, J., Arensburger, P., Pietrantonio, P.V., Barker, S.C., Shao, R., Zdobnov, E.M., Hauser, F., Grimmelikhuijzen, C.J.P., Park, Y., Rozas, J., Benton, R., Pedra, J.H.F., Nelson, D.R., Unger, M.F., Tubio, J.M.C., Tu, Z., Robertson, H.M., Shumway, M., Sutton, G., Wortman, J.R., Lawson, D., Wikel, S.K., Nene, V.M., Fraser, C.M., Collins, F.H., Birren, B., Nelson, K.E., Caler, E., Hill, C.A., 2016. Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. Nat. Commun. 7, 10507.

Haas, B.J., Gevers, D., Earl, A.M., Feldgarden, M., Ward, D.V., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S.K., Sodergren, E., 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res. 21, 494–504.

Hofmeister, E.K., Kolbert, C.P., Abdulkarim, A.S., Magera, J.M., Hopkins, M.K., Uhl, J.R., Ambyaye, A., Telford 3rd, S.R., Cockerill 3rd, F.R., Persing, D.H., 1998. Cosegregation of a novel *Bartonella* species with *Borrelia burgdorferi* and *Babesia microti* in *Peromyscus leucopus*. J. Infect. Dis. 177, 409–416.

Johnson, P.T.J., De Roode, J.C., Fenton, A., 2015. Why infectious disease research needs community ecology. Science 349.

Karesh, W.B.D., Andy, Lloyd-Smith, James O., Lubroth, Juan, Dixon, Matthew A., Bennett, Malcolm, Aldrich, Stephen, Harrington Pierre, T.F., Loh, Elizabeth H., Machalaba, Catherine C., Thomas, Mathew Jason, Heymann, David L., 2012. Ecology of zoonoses: natural and unnatural histories. Publ. Health Resour. Pap. 296.

Lackey, J.A., Huckaby, D.G., Ormiston, B.G., 1985. Peromyscus leucopus. Mamm. Species Am. Soc. Mammalogists 247, 1–10.

Ledevin, R., Millien, V., 2013. Congruent morphological and genetic differentiation as a signature of range expansion in a fragmented landscape. Ecol. Evol. 3, 4172–4182.

Leo, S.T., Gonzalez, A., Millien, V., 2016. Genetic signature of range expansion in a disease vector. J. Hered. http://dx.doi.org/10.1093/jherd/esw073.

Ley, R.E., Bäckhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., Gordon, J.I., 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. U. S. A. 102, 11070–11075.

Linnenbrink, M., Wang, J., Hardouin, E.A., Kunzel, S., Metzler, D., Baines, J.F., 2013. The role of biogeography in shaping diversity of the intestinal microbiota in house mice. Mol. Ecol. 22, 1904–1916.

Lozano, R., Naghavi, M., Foreman, K., et al., 2013. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. Lancet 380, 2095.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.
Lynch, M.F., Tauxe, R.V., Hedberg, C.W., 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol. Infect. 137, 307–315.
Maggi, R.G., Breitschwerdt, E.B., 2005. Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. J. Clin. Microbiol. 43, 1171–1176.
Mather, T.N., Wilson, M.L., Moore, S.I., Ribeiro, J.M.C., Spielman, A., 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). Am. J. Epidemiol. 130, 143–150.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8 (4).
Melzi, M.L., Ferrari, G.M., D'Adda, A., Bovo, G., Foresti, S., Cavallero, A., Biondi, A., 2015. Hepatic granulomatous lesions caused by systemic Bartonella vinsonii subsp. arupensis infection in a child. Pediatr. Infect. Dis. J. 34 (12), 1416–1417.
Minard, G., Tran, F.H., Van, V.T., Goubert, C., Bellet, C., Lambert, G., Kim, K.L., Thuy, T.H., Mavingui, P., Valiente Moro, C., 2015. French invasive Asian tiger mosquito populations harbor reduced bacterial microbiota and genetic diversity compared to Vietnamese autochthonous relatives. Front. Microbiol. 6, 970.
Moeller, A.H., Peeters, M., Ndjango, J.B., Li, Y., Hahn, B.H., Ochman, H., 2013. Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. Genome Res. 23, 1715–1720.
Nardis, C., Mosca, L., Mastromarino, P., 2013. Vaginal Microbiota and Viral Sexually Transmitted Diseases.
Ochman, H., Worobey, M., Kuo, C.-H., Ndjango, J.-B., Peeters, M., 2010. Evolutionary relationships of wild hominids recapitulated by gut microbial communities. PLoS Biol. 8 (11).
Ogden, N.H., Margos, G., Aanensen, D.M., Drebot, M.A., Feil, E.J., Hanincova, K., ... Lindsay, L.R., 2011. Investigation of genotypes of *Borrelia burgdorferi* in *Ixodes scapularis* ticks collected during surveillance in Canada. Appl. Environ. Microbiol. 77 (10), 3244–3254.
Oksanen, J.B., Guillaume, Kindt, Roeland, Legendre, Pierre, Minchin, Peter, O'Hara, R.B., Gavin, Simpson, L., Solymos, Peter, Henry, M., Stevens, H., Wagner, Helene, 2013. vegan: Community Ecology Package (R package version 2.0-9).
Ostfeld, R.S., 2011. Lyme Disease: The Ecology of a Complex System. Oxford University Press, p. 216.
Ostfeld, R.S., Levi, T., Jolles, A.E., Martin, L.B., Hosseini, P.R., Keesing, F., 2014. Life history and demographic drivers of reservoir competence for three tick-borne zoonotic pathogens. PLoS One 9, e107387.
Phillips, C.D., Phelan, G., Dowd, S.E., McDonough, M.M., Ferguson, A.W., Delton Hanson, J., Siles, L., Ordonez-Garza, N., San Francisco, M., Baker, R.J., 2012. Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. Mol. Ecol. 21, 2617–2627.
Radolf, J.D., Caimano, M.J., Stevenson, B., Hu, L.T., 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease *Spirochaetes*. Nat. Rev. Microbiol. 10, 87–99.
Rajaram, S., Oono, Y., 2010. NeatMap-non-clustering heat map alternatives in R. BMC bioinformatics 11 (1), 45.
Rogic, A., Tessier, N., Legendre, P., Lapointe, F.J., Millien, V., 2013. Genetic structure of the white-footed mouse in the context of the emergence of Lyme disease in southern Quebec. Ecol. Evol. 3, 2075–2088.
Roy-Dufresne, E., Logan, T., Simon, J.A., Chmura, G.L., Millien, V., 2013. Poleward expansion of the white-footed mouse *Peromyscus leucopus* under climate change: implications for the spread of Lyme disease. PLoS One 8, e80724.
Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., Walker, A.W., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12, 1–12.
Sanders, J.G., Beichman, A.C., Roman, J., Scott, J.J., Emerson, D., McCarthy, J.J., Girguis, P.R., 2015. Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores. Nat. Commun. 6, 8285.
Simon, J.A., Marrotte, R.R., Desrosiers, N., Fiset, J., Gaitan, J., Gonzalez, A., Koffi, J.K., Lapointe, F.-J., Leighton, P.A., Lindsay, L.R., Logan, T., Milord, F., Ogden, N.H., Rogic, A., Roy-Dufresne, E., Suter, D., Tessier, N., Millien, V., 2014. Climate change and habitat fragmentation drive the occurrence of *Borrelia burgdorferi*, the agent of Lyme disease, at the northeastern limit of its distribution. Evol. Appl. 7, 750–764.
R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org.
Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., Gordon, J.I., 2009. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci. Transl. Med. 1.
Walter, J., 2008. Ecological role of *Lactobacilli* in the gastrointestinal tract: implications for fundamental and biomedical research. Appl. Environ. Microbiol. 74, 4985–4996.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.
Weinstock, G.M., 2012. Genomic approaches to studying the human microbiota. Nature 489, 250–256.
Welch, D.F., C., K., Hofmeister, E.K., Persing, D.H., Robison, D.A., Steigerwalt, A.G., 1999. Isolation of a new subspecies, *Bartonella vinsonii* subsp. arupensis, from a cattle rancher: identity with isolates found in conjunction with *Borrelia burgdorferi* and *Babesia microti* among naturally infected mice. J. Clin. Microbiol. 37, 2598–2601.
Yun, Y., Srinivas, G., Kuenzel, S., Linnenbrink, M., Alnahas, S., Bruce, K.D., Steinhoff, U., Baines, J.F., Schaible, U.E., 2014. Environmentally determined differences in the murine lung microbiota and their relation to alveolar architecture. PLoS One 9, e113466.