Bacterial blood microbiome of rodents captured from a human/livestock/wildlife interface in Bushbuckridge, South Africa

AO Kolo[1], CA Gall[1,4], JM Wentzel[3], FB Kolo[2], H van Heerden[2], NE Collins[1], KA Brayton[1,4], MC Oosthuizen[1]

[1]Vectors and Vector-borne Diseases Research Programme, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa; [2]Bovine Tuberculosis and Brucellosis Research Programme Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa [3]Hans Hoheisen Wildlife Research Station, Faculty of Veterinary Science, University of Pretoria, South Africa; [4]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA; E-mail: afrianimalfriend@yahoo.co.uk

INTRODUCTION

Zoonotic pathogens make up an important and increasing number of emerging and reemerging infectious diseases of humans worldwide [1]. It has been documented that rodents serve as hosts and reservoirs of over 60 zoonotic pathogens that pose significant challenges to human health [2]. The Mnisi community area in Bushbuckridge Municipality, Mpumalanga Province, South Africa is cradled in the heart of a human/livestock/wildlife interface. In this community humans, domestic animals and wildlife have perennial direct and indirect contact. Research in the area has found rodents to be common and abundant [3] with 76% of households reported seeing rodents around their homes. Of that number 62% of the respondents saw them daily. A recent study in the area suggests that rodent-borne zoonoses may be implicated as causes of non-malarial acute febrile illness [4]. In this study, 6.5% of acute febrile illness patients tested positive for the rodent-borne zoonotic pathogen *Bartonella* spp. on PCR, while 6.8% of patients showed prior exposure to *Coxiella burnetti*, the cause of Q fever and 2.3% to *Leptospira* spp. [4]. The surveillance of zoonotic pathogens in rodents in this community is thus of utmost importance as the role they play in the transmission of zoonotic pathogens to humans is unknown.

AIM

The aim of this project was to provide a comprehensive insight into bacterial pathogens in the blood of wild rodents captured from different habitat areas in Mnisi using next-generation sequencing approaches.

METHODS

Barcoded sample-specific primers were used to amplify the 16S ribosomal RNA gene from genomic DNA from 25 wild rodents all previously molecularly identified as *Mastomys* spp. The rodents were collected from three habitat types which were (i) urban/periurban (Gottenburg/Hlalakahle), (ii) communal rangelands (Tlhavekisa) and (iii) protected area (Manyelethi) **(Figure 1)**. All PCR reactions with the same sample barcode were run in triplicate [5]. Purified PCR amplicons were submitted for circular consensus sequencing on the Pacific Biosciences platform at the genomic sequencing core of the Washington State University, Pullman, USA. Binning, trimming and analysis of sequence data was done using the CLC genomics workbench 8.5 (Qiagen), NCBI BLASTn command line application, the Ribosomal Database Program (RDP) 16S classifier [6] and Microsoft Excel. Principal Coordinate Analysis (PCOA) using the Bray-Curtis index [7] was done in the CLC microbial genomics module to quantify the compositional similarity or dissimilarity of the bacterial population in the blood of the rodents across the habitat areas. A phylogenetic tree of the 16S rRNA sequences from the dominant pathogen (i.e. *Bartonella* spp.) was constructed using the Maximum Likelihood method in the (MEGA7) software package.

RESULTS

Sequence analysis revealed a total of **65,062** bacterial sequences. The average number of reads per sample were 2,602 sequences which was sufficient to satisfy a rarefaction curve that all operational taxonomic units (OTUs) were captured. Sequences with less than 98% identity were assigned to genus level while those 98% and above were assigned to species level. Organisms that were not of zoonotic nor veterinary interest and were less than 1% of the total number of sequences were grouped as **'rare'**. Zoonotic pathogens of interest detected include ***Bartonella grahamii* (28.7%)**, ***B. tribocorum* (23%)**, unspeciated ***Bartonella* (12%)**, ***B. pachyuromydis***, ***B. phoceensis***, ***B. vinsonii* subsp. *arupensis***, ***B. elizabethae*** and ***B. rattimassiliensis***. Overall, rodents from Hlalakahle (urban/periurban) and Thlavekisa (communal rangeland) had higher proportions of *Bartonella* spp. (~85%), while Gottenberg (urban/periurban) and Manyelethi (protected area) (~45%) had lower *Bartonella* loads**.** Other organisms of zoonotic and veterinary significance detected included ***Ehrlichia chaffeensis*** (~0.03%), ***Anaplasma phagocytophilum*** (~0.5%), ***A. centrale*** (~0.01%) and members of the genus ***Brucella*** (~1%): ***Brucella abortus***, ***B. melitensis*** and ***B. ceti***. **(Figure 2)**. An ITS gene PCR and a multiplex AMOS PCR for *Brucella* spp. were used to confirm the presence of *Brucella abortus* DNA. We are still in the process of validating the detection of *E. chaffeensis*. Principal coordinate analysis showed similarities in bacterial populations in rodents from Thlavekisa and Hlalakahle. Rodents from Manyelethi had more conserved profiles in the bacterial community while rodents from Gottenberg showed the most diversity in their blood bacterial populations **(Figure 3).** Rodents from all habitat areas were found to have co-infections with different *Bartonella* spp. as seen on phylogenetic analysis of the 16S rRNA gene **(Figure 4).**

DISCUSSION

This serves as the first report of the detection of zoonotic bacterial pathogens: *Bartonella grahamii*, *B. tribocorum* and other *Bartonella* spp., as well as *Brucella* spp. and *Ehrlichia chaffeensis* in rodents in a human/livestock/ wildlife interface in South Africa. It highlights the role rodents play as reservoirs of these important organisms and underpins the risks posed to human health within the community.

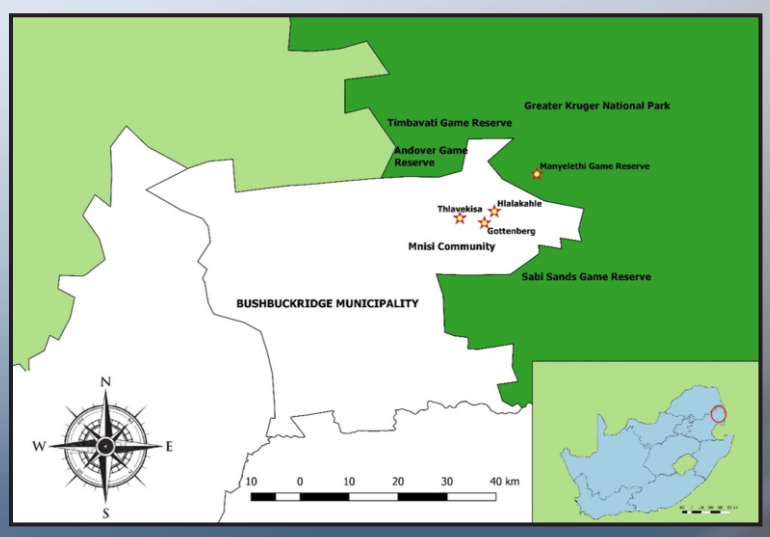

Figure 1: Map of Bushbuckridge Municipality, Mpumalanga Province South Africa. Stars indicate collection sites where rodents were captured. Dark green areas show the wildlife reserves surrounding the municipality.

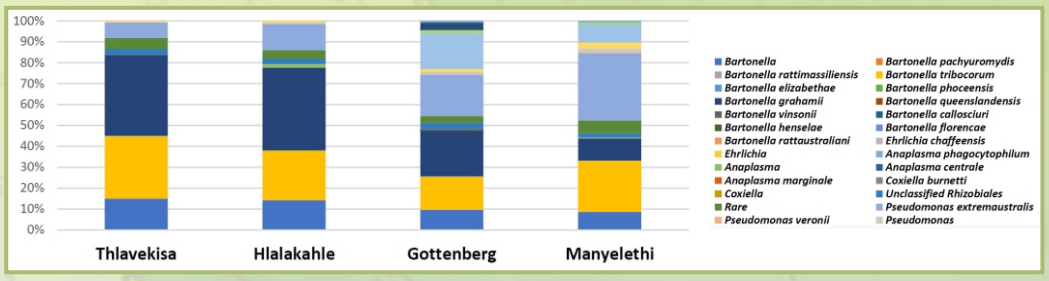

Figure 2: Stacked column chart showing the bacterial blood microbiome of wild rodents from different habitat areas in Bushbuckridge as revealed by 16S rRNA gene sequence analysis.

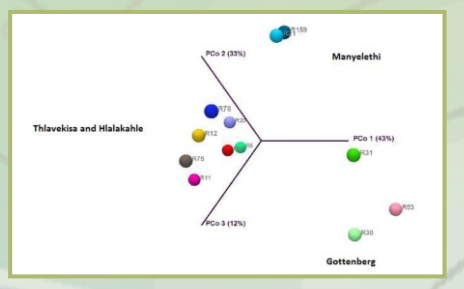

Figure 3: Principal Coordinate analysis with the Bray-Curtis index showing the similarities and dissimilarities within the bacterial populations in the blood of rodents from different habitat areas in Bushbuckridge.

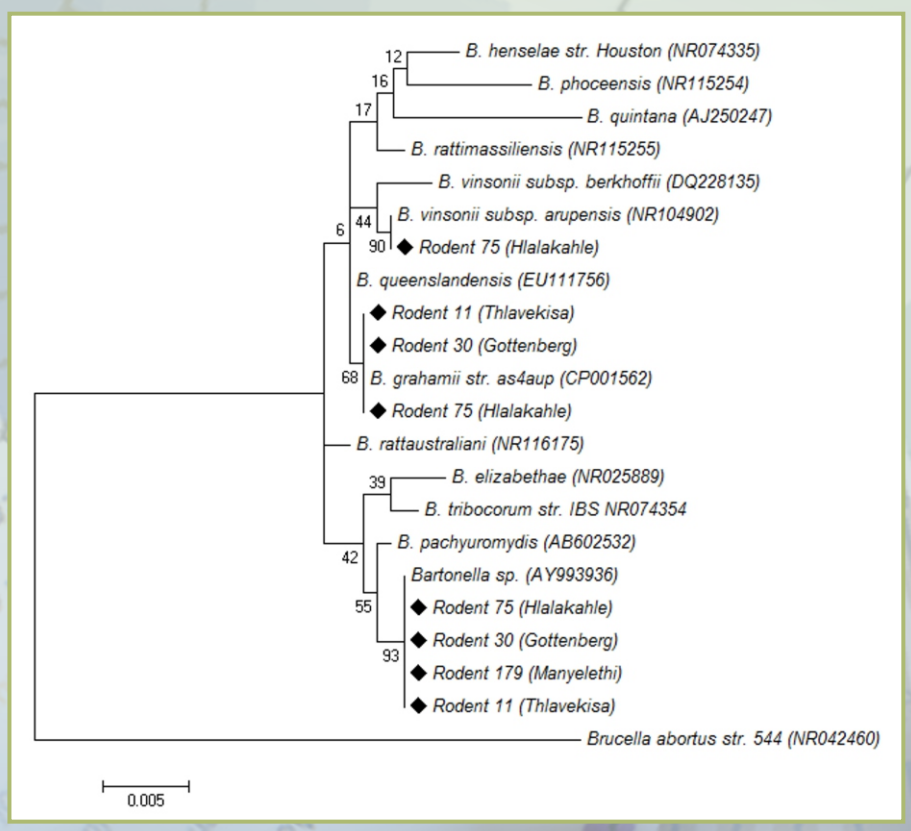

Figure 4: Maximum likelihood tree based on the Jukes-Cantor model showing the phylogenetic relationship between 16S rRNA gene sequences, previously identified *Bartonella* spp. and *Bartonella* spp. identified in in the blood of rodents from different habitat areas in Bushbuckridge. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 22 nucleotide sequences. There were a total of 1285 positions in the final dataset.

ACKNOWLEDGEMENTS

We would like to thank the funding agencies: Belgian Directorate General for Development Co-operation Framework (ITM/DGCD), and the South African National Research Foundation (grant 92739 to Marinda Oosthuizen). Prof Sonja Matthee (Stellenbosch University, South Africa) for providing expertise during the wild rodent trapping and Dr Armanda Bastos of the University of Pretoria for molecular identification of the rodents. Mark Wildung and Derek Pouchnik of the genomic sequencing core of the Washington State University, Pullman USA for technical assistance with Pacific Biosciences sequencing. The authors are grateful to Estelle Mayhew for the graphic design.

REFERENCES

1. Woolhouse, M.E.J. and S. Gowtage-Sequeria, Emerg Infect Dis, 2005. 11(12): p. 1842-1847.
2. Luis, A.D., et al., Proc Biol Sci, 2013. 280(1756): p. 20122753.
3. Berrian, A.M., et al., Prev Vet Med, 2016. 130: p. 119-128.
4. Simpson, G.J.G., et al., Vector Borne Zoonotic Dis, 2018. 18(6): p. 303-310.
5. Gall, C.A., et al., Isme j, 2016. 10(8): p. 1846-55.
6. Cole, J.R., et al., Nucleic Acids Res, 2009. 37(Database issue): p. D141-5.
7. Bray, J.R. and J.T. Curtis, 1957. 27(4): p. 325-349.

VECTORS AND VECTOR-BORNE DISEASES RESEARCH PROGRAMME
DEPARTMENT OF VETERINARY TROPICAL DISEASES

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa