Detection of zoonotic bacterial pathogens in various hosts in the Mnisi community, Mpumalanga, South Africa using a microbiome sequencing approach

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INTRODUCTION

The Mnisi community, an agro pastoral area adjacent to the Kruger National Park, Mpumalanga Province, South Africa, is classified as one of South Africa's 14 rural poverty nodes. It is nestled at the cusp of a human-livestock-wildlife interface (Fig 1). In this area, undifferentiated non-malarial acute febrile illness (AFI) is among the most common presenting sign in patients seeking healthcare at the community clinics[1,2]. Recent research suggested that zoonotic pathogens either rodent-borne or tick-borne may be common aetiologies of febrile illness in the community[1]. The study had shown that patients presenting with non-malarial AFI had prior exposure to *Bartonella* spp., spotted fever group *Rickettsia*, *Coxiella burnetti* and *Leptospira* spp. Low levels of West Nile and Sindbis, but no Rift Valley fever virus exposure were found. In a separate study, partial 16S rRNA gene sequences closely related to the zoonotic tick-borne rickettsial pathogen *Anaplasma phagocytophilum* have been detected in domestic dogs in the area and *R. africae* was found in ticks collected from dogs[3]. Research in the area has also found rodents to be common and abundant[4] with 76% of households reporting that they have seen rodents around their homes; of which 62% saw them on a daily basis. The active surveillance for potential pathogens in febrile patients, wild rodents, domestic dogs and cattle is thus of utmost importance in order to identify emerging zoonotic pathogens which could impact human health and livestock production in the Mnisi area and beyond.

AIM

The aim of this study was to investigate wild rodents, domestic dogs and cattle as possible sources of zoonoses using a microbiome sequencing approach.

METHODS

Barcoded sample-specific primers [5] were used to amplify the 16S ribosomal RNA gene from genomic DNA from nine AFI patients, 25 *Mastomys* rodent species, ten dogs and nine cattle. 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, NCBI BLASTn command line application, the Ribosomal Database Program (RDP) 16S classifier[6] and Microsoft Excel. For dogs and cattle data, sequences were subsequently blasted against a local database created from *Anaplasma* spp. sequences downloaded from GenBank to ascertain precise assignment of *Anaplasma* spp. sequences within the microbiome.

RESULTS

AFI patient microbiome (Fig 2a):

- 13,725 sequences
- The AFI patient blood microbiome was dominated *Rickettsia africae* (16%) (the cause of African tick-bite fever, detected in three of the nine AFI patients), as well as the opportunistic pathogens *Herbaspirillum huttiense* (27%) and *Stenotrophomonas maltophilia* (15.1%).
- The zoonotic bacterial pathogen *Brucella melitensis* was detected from one AFI patient.

***Mastomys* rodent microbiome (Fig 2b):**

- 65,060 sequences
- The rodent microbiome was dominated by *Bartonella* spp. (64%): *B. grahamii* (29%), *Bartonella* sp. strain RF255YX (23%), *Bartonella* spp. (12%).
- Other organisms of zoonotic and veterinary significance detected: *Bartonella henselae* (0.1%), *Ehrlichia* sp. (~0.03%), *Coxiella burnetti* (~0.02%), *Anaplasma* spp. (~0.5%), and *Brucella* spp. (~1%).
- Overall, rodents from Hlalakahle (urban/periurban) and Thlavekisa (communal rangeland) had higher proportions of *Bartonella* spp. (~85%), while those from Gottenberg (urban/periurban) and Manyeleti Game Reserve (wildlife area) (~45%) had lower *Bartonella* loads.

Dog microbiome (Fig 2c):

- 30,340 sequences
- The dog blood microbiome was dominated by *Ehrlichia canis* (24%), *Anaplasma platys* (19.3%), *Anaplasma* sp. ZAM dog (14.8%) and *Achromobacter xylosoxidans* (21.4%).
- *Achromobacter xylosoxidans* also made up 3.6% of the total sequences from the AFI patients. This could be an important finding since a previous study speculated that owners of dogs share a more similar bacterial microbiome with their pet dogs than with other dogs[7].
- Species represented by relatively small numbers of sequences in dogs included: *Mycoplasma haemocanis* (5%), *A. phagocytophilum* (0.3%), while 1.6% of the total sequences obtained from canine blood corresponded to other *Anaplasma* spp.

Cattle microbiome (Fig 2d):

- 34,559 sequences
- The cattle blood microbiome was dominated by *A. marginale* (58%), *Anaplasma* sp. Mymensingh (22.2%), *Anaplasma* spp. (10.5%) and *Anaplasma* sp. Dedessa (5.4%).
- Other species of interest: *A. centrale* (1.4%), *Bartonella* spp. (0.5%), *A. platys* (0.2%) and *A. phagocytophilum* (0.01%). Very few sequences of *Borrelia* sp., *Brucella* sp., *Bartonella bovis* and the novel pathogen *Ehrlichia minasensis* were also detected.

DISCUSSION

This study detected an array of zoonotic bacterial pathogens: *B. grahamii*, *B. henselae*, *C. burnetti*, *R. africae*, *Brucella* spp., *A. platys* and *A. phagocytophilum* and highlights their significance as potential contributing factors to non-malarial febrile illness in the Mnisi community area. We recommend that health care practitioners in the community should consider these pathogens in the differential diagnosis of non-malarial AFI, which will help to guide appropriate treatment.

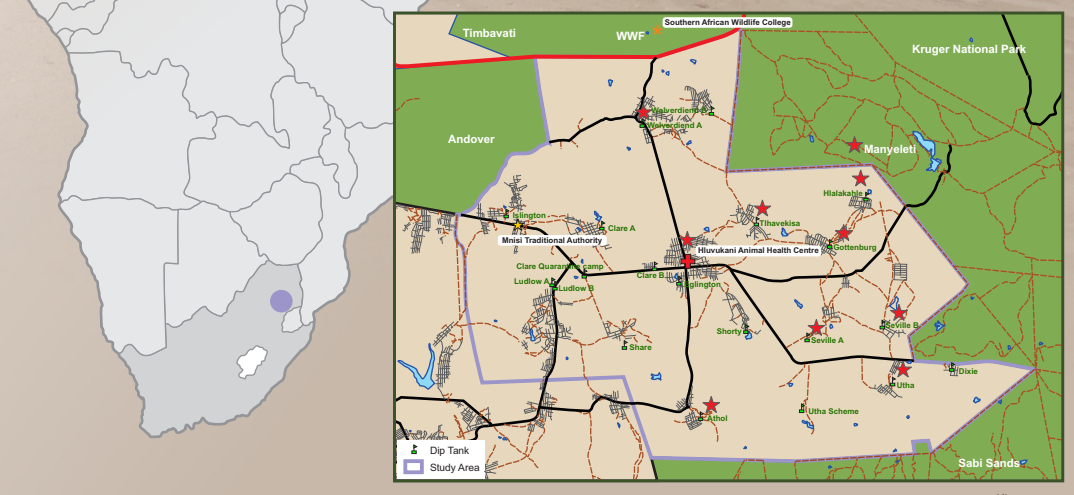

Figure 1: Map of the Mnisi community area, Bushbuckridge Municipality, Mpumalanga, South Africa[4]

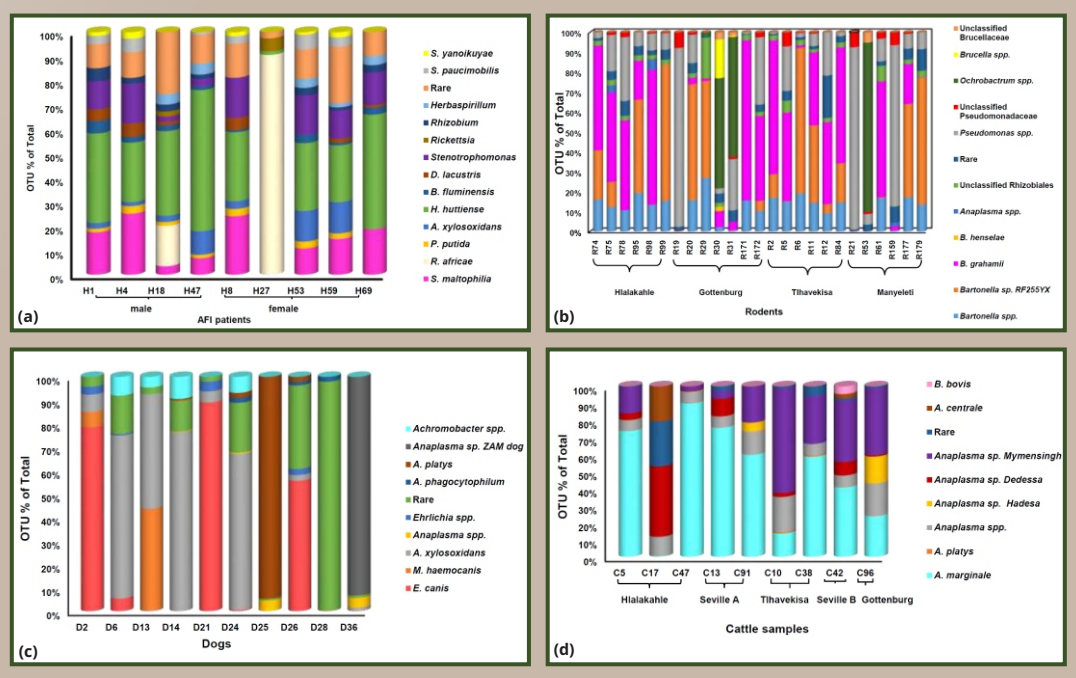

Figure 2: Relative abundance of the major taxa of bacteria in the blood of (a) nine AFI patients, (b) 25 *Mastomys* rodents captured across three habitat types (urban/periurban: Gottenburg/Hlalakahle; communal rangelands: Tlhavekisa; wildlife area: Manyeleti), (c) ten dogs and (d) nine cattle (sampled at five dip tanks) in the Mnisi community.

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