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Dynamics of Rodent-Borne Zoonotic Diseases and Their Reservoir Hosts: Invasive *Rattus* in South Africa

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ABSTRACT: Lack of adequate sanitation and pest control, and poor housing conditions that prevail in much of both rural and urban South Africa, cause rodent populations to thrive, promoting contact with humans, which results in increased risk of zoonotic disease transmission. This study focused on bacterial pathogens involved in potential zoonoses present in 3 commensal and invasive *Rattus* spp., namely *Rattus norvegicus*, *R. rattus*, and *R. tanezumi*, from rural and urban South Africa. Bacterial prevalence and diversity was determined through amplification and sequencing of the mitochondrial 16S gene region, using 4 primer sets: 2 that have a broad bacterial species recognition range, and 2 genus-specific sets that target the genera *Streptococcus* and *Streptobacillus*. An overall bacterial prevalence of 32% ($n = 84$) in kidney samples was obtained using the 16S universal primer sets. Subsequent sequence analyses found bacterial prevalence per host species to be 41% for *R. norvegicus*, 42% for *R. rattus*, and 8% for *R. tanezumi*, with representatives of diverse bacterial taxa such as *Clostridium sordelli* (toxic shock syndrome), *Bacillus cereus* (diarrhoeal disease), and *Enterococcus faecalis* (nosocomial infections) being characterised. The primer set targeting *Streptobacillus moniliformis* was used to determine the prevalence of this zoonotically-important bacterial taxon, which is transmissible via a bite from *Rattus* to humans, whilst *Streptococcus*-specific primers were used to assess environmental shedding of this agent via the urinary route. The results highlight the public health implications especially for immune-compromised individuals, as these rodent-borne pathogens can cause opportunistic infections that in such individuals are not readily diagnosed or treated.

KEY WORDS: bacteria, *Bartonella*, *Clostridium*, disease, prevalence, public health, *Rattus norvegicus*, *Rattus rattus*, *Rattus tanezumi*, South Africa, *Streptobacillus*, *Streptococcus*, zoonoses

INTRODUCTION

Three species of invasive commensal *Rattus*, namely *R. norvegicus*, *R. rattus*, and *R. tanezumi*, are known to occur in South Africa, the latter being only recently identified in South Africa (and the African continent) in 2005 using a genetic approach (Bastos et al. 2011). *Rattus tanezumi* and *R. rattus* are cryptic species that form part of the *Rattus* sp. complex (Taylor et al. 2008, Bastos et al. 2011), and although often occurring in sympatry cannot be distinguished from each other morphologically (Musser and Carleton 2005, Mostert 2010). In contrast, *R. norvegicus* is readily distinguished by its significantly larger body size and external morphological features. These invasive rodents are known as commensal species (Atkinson 1985, Taylor et al. 2008, Bastos et al. 2011), as they habitually congregate with humans and exploit human habitation for food and shelter. The lack of adequate sanitation and pest control, and the poor housing conditions that prevail in much of rural and urban South Africa (Oldewage-Theron et al. 2006, Taylor et al. 2008), result in thriving rodent populations, promoting contact with humans (Gratz 1999) and increased risk of zoonotic disease transmission.

Rodents of the genus *Rattus* play a significant role in the transmission of zoonotic diseases (Gratz 1997). Several hanta- and arena viruses known to cause haemorrhagic fevers (Mills and Childs 1998), bacterial pathogens (Azad and Beard 1998, Bermond et al. 2000, Breitschwerdt and Kordick 2000), as well as helminths and protozoan parasites (Claveria et al. 2005) have been isolated from rats. In addition, rats may harbour several ectoparasites that can act as vectors capable of transmitting pathogens to humans (Carter and Cordes 1980, Azad and Beard 1998, Yang et al. 2009). There are also various routes of transmission, either directly through rat bites/scratches, or indirectly through environmental contamination by rat urine/faeces or through ectoparasite vectors.

Streptobacillus moniliformis is a bacterial pathogen that can be directly and indirectly transmitted to humans via a rat bite, scratch, or through contact with any excreta (saliva, urine faeces) (Gaastra et al. 2009). The pathogen is considered to be part of the natural flora in the nasopharynx of rats (Strangeways 1933, Wullenweber 1995), and rats are potential reservoir hosts, as infected individuals often appear healthy and asymptomatic (Elliott 2007). More importantly, *S. moniliformis* is one of the causative agents of rat bite fever, a rare zoonotic infection that is most likely under-reported (Gaastra et al.

2009) and misdiagnosed (Freels and Elliott 2004) due to non-specific presentation. Prior to 2000, information was limited to individual case reports, despite the etiological agent having been discovered nearly a hundred years earlier (Elliott 2007).

Consideration of this pathogen is vital for South African human health as commensal *Rattus* are frequently associated with rat bites in urban and rural areas of South Africa (Kirsten and von Maltitz 2005). Children (e.g., Zwecker 2010, Anonymous 2011) and persons of low socio-economic status, as is the case in other parts of the world, are typically affected (Wullenweber 1995, Hirschorn and Hodge 1999). Worsening the problem in South Africa is large-scale rodent control initiatives that are reactive instead of proactive. In contrast to other parts of the world, no clear rodent control guidelines or management protocols exist in South Africa (Fritts 2007).

Given the above information, the aim of this study was to obtain a preliminary assessment of the diversity of the bacterial community of 3 commensal *Rattus* species that are of zoonotic and public health importance in South Africa.

MATERIALS AND METHODS
Sampling
Rats (*Rattus* spp.) were obtained by a combination of captures and donations from members of the public and pest control companies. Rats were trapped using Sherman traps (H.B. Sherman Inc., Tallahassee, FL) as well as snap traps (Scientific Supa-Kill, Kempton Park, South Africa). Samples were collected from 18 localities in 4 provinces (Gauteng, Kwa-Zulu Natal, Limpopo, and Mpumalanga) in South Africa during the periods 2003-2004 and 2010-2011. Sampling locations were representative of urban, semi-urban, and rural areas. For the 2010-11 sampling, approximately 50 snap traps and 100 Sherman traps were baited with a peanut butter, fish, and oatmeal mixture and placed in and around storage facilities, office buildings, and human dwellings. Traps were inspected daily for a trapping period of one week per month. All live-trapped rats were transported to the laboratory at the Department of Zoology and Entomology, University of Pretoria, for subsequent euthanisation and examination, while snap trapped individuals were individually bagged and transported at 4ºC to the same laboratory.

Laboratory Procedures
Where live animals were sampled, these were maintained under the guidelines of the American Society of Mammalogists (ASM 2011) and as approved by the Animal Care and Use Committee of the University of Pretoria. Live animals were euthanised by means of halothane inhalation, standard measurements were recorded, and oral swabs (*n* = 70) were collected and stored in 1:1 PBS/glycerol solution while kidney tissues (*n* = 84) were removed and preserved at -20ºC for subsequent molecular analysis. Voucher specimens will be prepared and deposited in the small mammal reference collection of the Ditsong Natural Museum of Natural History, Pretoria, South Africa. Genomic DNA was extracted from all oral swabs and kidney tissue using the Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Randburg, South Africa) according to the manufacturer's protocol, and were stored at -20ºC.

Host Species Identification
Using kidney/oral swab DNA extracts, amplification of mitochondrial cytochrome b (cyt b) gene region was performed with the primer set L14724-TGAYATGAAAAAYCATCGTTG and H15915- CATTTCAGGTTTACAAGAC (1.2 kbp amplicon) (Bastos et al. 2005, Mostert 2010) in order to molecularly differentiate between the two cryptic species R. rattus and R. tanezumi, and to confirm identification of juvenile R. norvegicus. The reaction conditions were as follows: initial denaturation at 96ºC for 20 s, followed by two cycles of denaturation at 96ºC for 12 s, annealing at 49ºC for 25 s, and extension at 72ºC for 60 s, five cycles of denaturation at 96ºC for 12 s, annealing at 47ºC for 20 s, and extension at 72ºC for 55 s, and finally 35 cycles of denaturation at 96ºC for 12 s, annealing at 45ºC for 15 s, and extension at 72ºC for 50 s, with a final extension at 72ºC for 1 minute. All adult individuals of R. norvegicus were identified based on their significantly large body size and external morphological features, different to that of the two congeners.

Bacterial Identification
Kidney DNA extracts were screened for overall bacterial prevalence using one of the two universal/broad-range forward primers viz 27F- AGAGTTTGATCCTGGCTCAG or 63F-CAGGCCTAACACATGCAAGTC) in combination with the reverse primer 1522R-AAGGAGGTGATCCAGCCGCA (Hauben et al. 1997, Marchesi et al. 1998). Each primer combination amplified a region of approximately 1.5kbp of the 16S rRNA gene region. Amplification was achieved using a touchdown PCR in which an initial denaturation at 96ºC for 10 s was followed by three discrete thermal cycles, consisting of denaturation at 96º C for 12 s, annealing for 20 s, and extension at 70ºC for 1 min 50 s, with an initial annealing temperature of 63ºC, followed by 61ºC, and finally 32 cycles at 59ºC.

In addition, a genus-specific primer set, amplifying a 1 kbp section of the 16S gene of diverse Streptococcus species, was used to screen Rattus kidney samples, using the same approach detailed previously for assessing invasive Mus in the sub-Antarctic (de Bruyn et al. 2008). A touch-down PCR similar to that of the broad-range PCR thermal profile was used. Oral swab DNA extracts were screened using a Streptobacillus-specific primer set, S5- CATACTCGGAATAAGATGG and AS2- GCTTAGCTCCTCTTTGTAC, amplifying 269 bp of the 16S rRNA gene region (Kimura et al. 2008). A touch-down PCR method was again used to improve reaction specificity.

Microbial Culturing and Isolation
In order to identify S*treptococcus*-positive samples to species level, microbial culturing was attempted. Kidney tissue was suspended in brain-heart infusion broth and incubated at 37ºC for 16 hours under anaerobic conditions, and thereafter was inoculated on blood agar plates for 24 hours under similar conditions. Identification of isolated colonies was achieved using the broad-range PCR detailed earlier.

Phylogenetic and Statistical Analyses

All amplified PCR products were purified using the Roche PCR Product Purification Kit (Roche Diagnostics) and cycle sequenced using BigDye v. 3.1 terminator cycle-sequencing kit (Perkin-Elmer, Foster City, CA). Samples were run on an ABI 3130 sequencer and the resulting sequence chromatograms were viewed and edited in Mega 5 (Tamura et al. 2011), prior to performing a BLAST nucleotide database search (www.ncbi.nm.nih.gov/blast) to identify the species with the highest sequence similarity.

Differences in bacterial prevalence between host species, host sex, and host trap locality were analysed in STATISTICA v10 (StatSoft Inc., Tulsa, OK) using a one-way analysis of variance (ANOVA), with host species as the fixed factor and a Tukey test for multiple comparisons between treatments.

RESULTS
Host Species Identification and Distribution

Mitochondrial-based and morphological host identification indicated that of the total of 124 rats captured, 48% were *R. norvegicus*, whereas *R. rattus* and *R tanezumi* represented 27% and 25% of captures, respectively. *Rattus norvegicus* was caught predominantly around human dwellings in informal settlements, while *R. rattus* and *R. tanezumi* were caught in residential areas, office buildings, and semi-urban small holdings. Although all 3 species did not occur sympatrically, 2 species, namely *R. rattus* and *R. tanezumi*, were found to co-occur at 2 localities (Moreleta Park and Hammanskraal) in the Gauteng Province (Figure 1) (Bastos et al. 2011).

Broad-Range Bacterial Primer Sets

Out of a total of 124 samples, 84 kidney samples were screened, and PCR amplification revealed an overall bacterial prevalence of 32%. Host species showed significant differences in bacterial prevalence ($F_{2,81}$ = 4.70, n = 84, P = 0.01) with *R. norvegicus* (41%) and *R. rattus* being similar (42%), while *R. tanezumi* (8%) had lower bacterial prevalence compared with the other 2 congeners. Species of the genus *Streptococcus* (n = 5) were the most prevalent bacteria, followed by *Bartonella* sp. (n = 3), *Clostridium sordelii* (n = 1), and *Acinetobacter* sp. (n = 1) (Figure 2). Furthermore, many mixed sequences (n = 17) were recovered. Although host genome amplification by 63F/1522R primer set has not been reported previously, 20 amplicons (1.5kbp) were found to correspond to the host genome. These samples were not considered for bacterial prevalence estimates.

Genus-Specific Primer Sets

The *Streptococcus*-specific primer set recovered four additional (to those recovered using broad-range primer sets) *Streptococcus* spp.-positive samples (n = 84), and sequence analyses revealed four previously undescribed species. Microbial culturing techniques, however, failed to isolate *Streptococcus* species from the kidney samples, and cultures of *Bacillus cereus* and *Enterococcus faecalis* were obtained instead (Table 1).
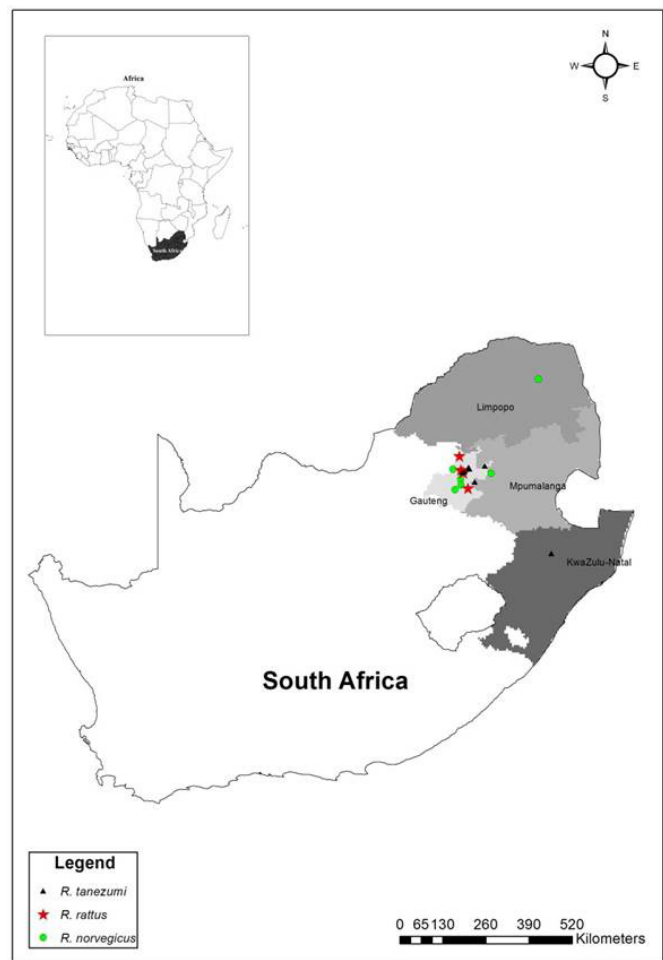

Figure 1. Map of South Africa indicating Gauteng, Kwa-Zulu Natal, Limpopo, and Mpumalanga provinces from which the 3 invasive *Rattus* species were collected.
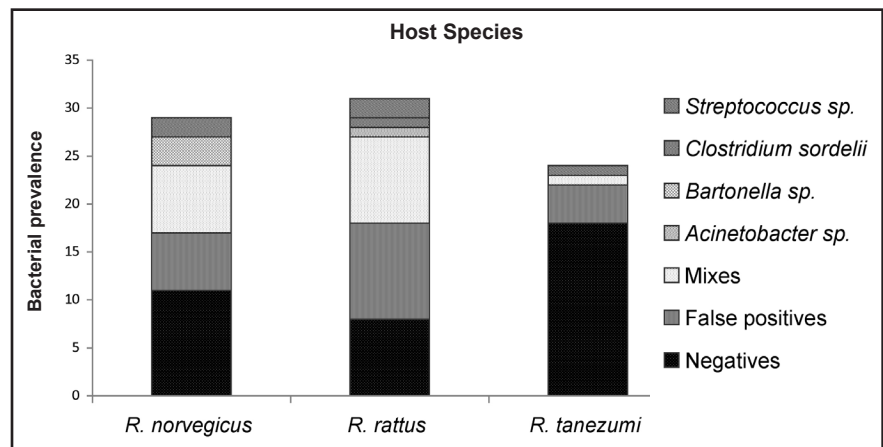

Figure 2. Bacterial prevalence in kidney samples of the 3 invasive *Rattus* species collected from rural and urban South Africa, indicating molecular prevalence within and between 3 host species.

Table 1. Microbial culturing and PCR results obtained from 9 *Streptococcus*-infected kidney samples of 3 *R. norvegicus* (Rn; *n* = 29), 3 *R. rattus* (Rr; *n* = 31) and 3 *R. tanezumi* (Rt; *n* = 24) species from 5 sample localities in South Africa. (+) and (-) denotes positive and no amplification.

Sample name	Host species (*Rattus*)	Sampling locality*	16S primer set	Genus specific primer set	Culture
			PCR		
ER5	*R. norvegicus*	Tembisa	+	+	no growth
HK26	*R. rattus*	Hammanskraal	-	+	*Bacillus cereus*
HSYR4	*R. tanezumi*	Hammanskraal	+	-	no growth
JZ11	*R. norvegicus*	JHB Zoo	+	-	no growth
JZ7	*R. norvegicus*	JHB Zoo	n/a	+	*Enterococcus faecalis*
UPER22	*R. rattus*	UP Exper. Farm	+	-	*Bacillus cereus*
UPER23	*R. rattus*	UP Exper. Farm	+	-	no growth
WK01	*R. tanezumi*	Waterkloof	-	+	no growth
WK02	*R. tanezumi*	Waterkloof	-	+	no growth

*JHB Zoo – Johannesburg Zoological Gardens; UP Exper. Farm – University of Pretoria Experimental Farm
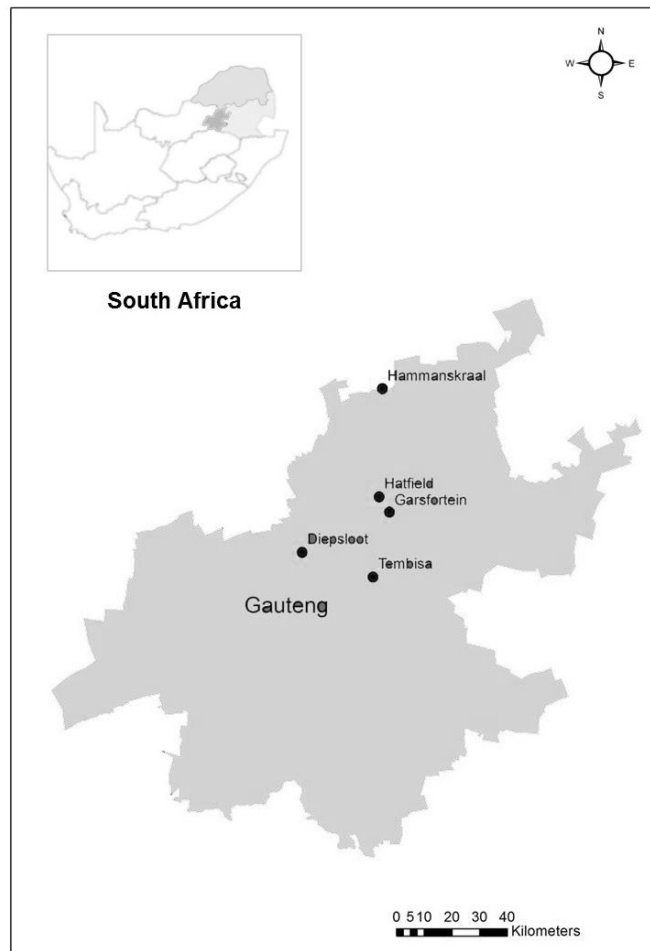

Figure 3. Distribution of *Streptobacillus moniliformis*-infected rats in Gauteng Province, South Africa.

A subset of 70 oral swab samples (all from Gauteng Province) was screened for *Streptobacillus moniliformis* presence. Overall prevalence was 30%, whereas rates of infection by species was 22% for *R. norvegicus*, 33% for *R. rattus*, and 46% for *R. tanezumi*; however, these differences were not statistically significant ($F_{2, 67}$ = 1.38, *n* = 70, *P* = 0.26). Geographical analyses of infection prevalence in Gauteng Province mirrored that of the host-species, as the sampling localities of the different host species were geographically distinct (Figure 3); *Rattus tanezumi* (*n* = 13) was captured in Hammanskraal, *R. rattus* (*n* = 21) was captured in Hatfield/Garsfontein, and *R. norvegicus* (*n* = 36) was captured in Tembisa/Diepsloot in the Gauteng Province. While 40% of females (*n* = 30) and only 23% of males (*n* = 40) were infected, the difference was not statistically significant ($F_{1, 68}$ = 2.52, *n* = 70, *P* = 0.18).

DISCUSSION

It has long been recognized that rats carry disease (Elliott 2007). In South Africa, however, very few studies (Gratz 1999, Taylor et al. 2008, Mostert 2010) have addressed the incidence of zoonotic diseases occurring in invasive *Rattus*, in particular. It is therefore of concern that all the bacterial genera recovered in this initial assessment are implicated in human disease and considered to be agents of zoonotic importance.

The *Bartonella* genus is known to cause severe endocarditis in immune-compromised individuals, while *Streptococcus* sp. may cause meningitis, nephritis, and systemic infection in humans (Breitschwerdt and Kordick 2000, Ruoff and Bisno 2010). *Acinetobacter* sp. is a soil and water pathogen, and although widely distributed in nature, can together with *Enterococcus faecalis* (a commensal in mammalian gut) become problematic in nosocomial environments (Solheim et al. 2009, Allen and Hartman 2010). *Clostridium sordelii* and *Bacillus cereus* are also soil pathogens, and *C. sordelii* has been linked to toxic shock syndrome in previously healthy individuals (CDC 2010) whilst *B. cereus* may cause food poisoning resembling that caused by *Staphylococcus* infection. In one extreme case, *B. cereus* caused fatal pneumonia in an intensive care unit patient (Fekete 2010).

Furthermore, both the high incidence of mixed sequences and contrasting culture and PCR results

revealed a high incidence of bacterial co-infection, emphasizing the abundance and diversity of the bacterial community within *Rattus* spp. As these bacteria were identified from kidney samples, transmission through environmental contamination by urine/faeces of rats is possible and of concern.

The presence of *Streptobacillus moniliformis* in commensal rats represents the first known record of this etiological agent in invasive *Rattus* in South Africa. This is of particular concern, as the disease is poorly understood (Freels and Elliott 2004, Gaastra et al. 2009). Although statistical analyses revealed no significant differences in infection prevalence between host species, gender, or trap locality, these results are suggestive of *S. moniliformis* infection being widely distributed, and this may be important in risk assessment. Rat bite incidence is high, particularly in rural communities. A survey in the Limpopo Province by Kirsten and von Maltitz (2005) indicated that rodent bites occur frequently, as 69% of respondents had experience with this. The high frequency of rat bites increases the potential transmission of rat bite fever to humans, and the disease is likely to be misdiagnosed and underreported, as it is a non-communicable and non-reportable disease with non-specific presentation (Gaastra et al. 2009).

The potential of rats to transmit disease indirectly through environmental contamination and directly via rat bites or scratches is evident from the literature. The identification of a wide range of bacterial genera with known zoonotic potential, in invasive *Rattus* in South Africa, stresses the need for further research on rodent-borne zoonotic diseases in rural and urban South Africa, and for the implementation of proactive pest management strategies.

ACKNOWLEDGEMENTS

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