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Rickettsia diversity in southern Africa: A small mammal perspective

Sandra Essbauer[a,*], Mirja Hofmann[a], Christoph Kleinemeier[a], Silke Wölfel[a], Sonja Matthee[b]

[a] Bundeswehr Institute of Microbiology, Department Virology and Rickettsiology, Neuherbergstr. 11, 80937 Muenchen, Germany
[b] Department of Conservation Ecology and Entomology, Stellenbosch University, Matieland, South Africa

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ABSTRACT

Worldwide, including Africa, rickettsioses are recognized as emerging or re-emerging infections. To date, little is known about the diversity of *Rickettsia* species that are naturally associated with small mammals in southern Africa. The aim of the study was to screen a diversity of small mammals for the presence of rickettsial DNA. Animals were trapped at 38 localities in South Africa and Namibia. In total, 1616 ear-tissue samples from 23 species representing 17 genera were tested using real-time (rt)PCR and multi-locus sequence typing (MLST). Of the 1616 samples 251 (15.5%) were positive in an initial rtPCR. In 16 of the 23 investigated animal species rickettsial DNA was detected with an average prevalence of 15.7%. We herein describe for the first time four *Rickettsia* (R.) species known to be pathogenic for humans in rodents from South Africa, *R. conorii, R. massiliae, R. felis* and *R. helvetica*. In addition, by MLST and subsequent phylogenetic analyses so far undescribed *Rickettsia* species, *Candidatus* Rickettsia africaustralis, *Candidatus* Rickettsia rhabdomydis, and *Candidatus* Rickettsia muridii were confirmed. Further four new genotypes, genotype Rickettsia hofmannii, genotype Rickettsia stutterheimensis, genotype Rickettsia hogsbackensis and genotype Rickettsia kaalplaasensis, respectively, are described. The data indicate a surprisingly high diversity of *Rickettsia* in small mammals in South Africa and might indicate their possible role as reservoirs for *Rickettsia*. Ecological questions concerning their natural hosts such as small mammals, but also the role of livestock or pet animals, require further investigation. Particularly, data on the relevance of these rickettsiae for diseases in humans are of further interest.

1. Introduction

The potential of *Rickettsia* to cause disease in humans has been known for more than 110 years. However, only with the advent of modern molecular techniques scientists have become aware of the extent and diversity of *Rickettsia* (Parola et al., 2013). In recent years a lot of new *Rickettsia* species have been described by multi-locus sequence typing (MLST) of four to five sequences and isolating of the respective species. Further several Candidatus species, i.e. defined by genotyping of for four or five fragments without isolation of the respective *Rickettsia* as well as a few genotypes, i.e. *Rickettsia* for which less than four or five gene fragments are available, have been described (Fournier et al., 2003; Raoult et al., 2005; Fournier and Raoult, 2009; Merhej et al., 2014). Despite their emergence as zoonotic diseases, rickettsioses can still be regarded as neglected diseases (Chikeka and Dumler, 2015). In general, rickettsiae are transmitted by various arthropods that may act as reservoirs and/or as vectors to humans and animals. Within the genus *Rickettsia* (*R.*), the typhus group, the spotted fever group (SFG) and *R. helvetica* are of medical importance (Parola et al., 2005; Merhej et al., 2014). These have been found in several countries in Africa, but systematic studies are lacking in most areas of sub-Saharan Africa thus far (Parola, 2006; Fournier and Raoult, 2009; Parola et al., 2013).

In South Africa, several further *Rickettsia* species associated with disease in humans have been recorded: *R. conorii* (SFG, McNaught, 1911), *R. aeschlimannii* (SFG, Beati et al., 1997; Pretorius and Birtles, 2002), *R. sibirica* subsp. (Pretorius and Birtles, 2004) and *R. africae*, causative agent of African tick bite fever (ATBF, Jensenius et al., 2003). In addition, recent studies described *R. massiliae* in ticks from tortoises (Halajian et al., 2016) and *R. felis* in ticks from dogs (Kolo et al., 2016). *R. africae* has often been implicated in ATBF in travelers returning from South Africa and Swaziland (Jensenius et al., 1999; Raoult et al., 2001; Pretorius et al., 2004; Parola et al., 2005; Büchau et al., 2006; Roch et al., 2008; Tappe et al., 2009; Althaus et al., 2010; Wieten et al., 2011; Beltrame et al., 2012; Schleenvoigt et al., 2012; Socolovschi et al., 2012). Also, *R. sibirica mongolitimonae* was confirmed in a patient suffering from lymphangitis, headache, and fever in South Africa (Parola, 2006). In Namibia thus far, only few serological studies in the indigenous population and in travelers returning to Europe are available (Wessels et al., 1986; Jensenius et al., 2003; Noden and van der Colf, 2013). Research on rickettsial vectors in South Africa includes reports

* Corresponding author.
E-mail address: sandraessbauer@bundeswehr.org (S. Essbauer).

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Table 1
Proportion of Rickettsia (partial *gltA* rtPCR)- positive samples.

A) In rodent species

Species		[% (number positive/ total number of samples)]
Rodents		
Rhabdomys spp.	four-striped mice	15.7 (192/1223)
Micaelamys namaquensis	Namaqua rock mice	17 (17/99)
Mastomys coucha	Southern multimammate mice	9 (6/64)
Mus musculus	house mice	5 (3/61)
Otomys irroratus	Southern African vlei rats	15 (5/33)
Myotomys unisulcatus	bush vlei rats	33 (7/21)
Lemniscomys rosalia	single-striped grass mice	60 (9/15)
Mastomys natalensis	Natal multimammate mice	0 (0/12)
Mus minutoides	African pygmy mice	0 (0/11)
Saccostomus campestris	South African pouched mice	0 (0/11)
Steatomys pratensis	fat mice	0 (0/11)
Aethomys chrysophilus	red rock rats	44 (4/9)
Rattus rattus	black rats	17 (1/6)
Desmodillus auricularis	cape short-eared gerbils	20 (1/5)
Gerbillurus paeba	hairy-footed gerbil	0 (0/1)
Insectivores		
Crocidura mariquensis	swamp musk shrews	20 (2/10)
Crocidura flavescens	greater musk shrews	0 (0/9)
Myosorex varius	forest shrews	17 (1/6)
Macroscelides proboscideus	round-eared elephant shrews	33 (1/3)
Crocidura cyanea	reddish-gray musk shrews	50 (1/2)
Myosorex cafer	dark-footed mouse shrews	50 (1/2)
Elephantulus rupestris	western rock elephant shrew	0 (0/1)
Crocidura bicolor	bicolored shrew	0 (0/1)
Sum		15.5 (251/1616)

B) at trapping localities

Localities	Provinces	No. in Fig. 1	[% (number positive/total number of samples)]
South Africa:			
Alice	ECP	1	10 (3/31)
Anysberg	WCP	2	42 (22/52)
Beaufort West	WCP	3	11 (4/37)
Caledon	WCP	4	0 (0/1)
Drie susters	WCP	5	22 (5/23)
East London	ECP	6	8.8 (10/114)
Elsenburg	WCP	7	10.2 (11/108)
Fort Beaufort	ECP	8	5 (3/62)
Groblershoop	NCP	9	0 (0/8)
Hogsback	ECP	10	15 (11/73)
Hottentots Holland	WCP	11	23 (17/73)
Stellenbosch 1	WCP	12	15 (2/13)
Jonkershoek	WCP	13	21.8 (24/110)
Kaalplaas	GP	14	20 (12/60)
Kimberley	NCP	16	6 (3/52)
Loeriesfontein	NCP	17	0 (0/4)
Loskop Dam Nature Reserve	MP	18	5 (1/20)
Stellenbosch 2	WCP	20	13 (4/32)
Mooinooi	NWP	21	24 (15/63)
Stellenbosch 3	WCP	22	5 (4/88)
Oribi Gorge	KZN	23	100 (1/1)
Oudtshoorn	WCP	24	7 (2/31)
Porterville	WCP	25	0 (0/25)
Richtersveld	NCP	26	2 (1/65)
Rietvlei Nature Reserve	GP	27	45 (23/51)
Somerset West	WCP	28	50 (4/8)
Springbok	NCP	29	12.8 (16/125)
Stellenbosch 4	WCP	30	25 (1/4)
Stutterheim	ECP	31	33 (1/3)
Swellendam	WCP	32	50 (1/2)
Tankwa Karoo National Park	NCP	33	50 (1/2)
Vanrhynsdorp	WCP	34	3 (1/30)
Vernon Crookes	KZN	35	100 (1/1)
Wellington	WCP	36	18.9 (23/122)
Wolwedans	WCP	37	48 (11/23)
Kuilsrivier	WCP	38	18 (13/73)
Namibia:			
Keetmanshoop		15	0 (0/22)
Mariental		19	0 (0/4)
Sum		38	15.5

Abbreviations: ECPEastern Cape Province ; GPGauteng Province: MP Mpumalanga ; NWPNorth-West Province ; NCPNorthern Cape Province ; WCPWestern Cape Province.

about *R. africae* detected in *Amblyomma* (*A.*) *hebraeum* (Kelly and Mason, 1991; Halajian et al., 2016; Kolo et al., 2016) and *Haemaphysalis* (*Hae.*) *elliptica* (Kolo et al., 2016). In addition, *R. aeschlimannii* has been found in *Rhipicephalus* (*R.*) *appendiculatus* (Pretorius and Birtels, 2002), *R. sibirica mongolitimonae* in *Hyalomma* (*H.*) species (Parola et al., 2001) and *R. massiliae* in *A. sylvaticum* (Halajian et al., 2016).

Apart from limited data on rickettsioses, investigations on the ecology of SFG rickettsiae e.g. the role of vector-pathogen-interactions, natural reservoirs and the factors that perpetuate rickettsiae dynamics in nature (e.g. Bozeman et al., 1967; Labruna, 2009) are rare. In Africa, there have been some investigations on pets, livestock animals and/or ectoparasites collected from these animals (Morita et al., 2004; Mediannikov et al., 2012; Kamani et al., 2013; Mutai et al., 2013; Hornok et al., 2014) or from small mammals (Berrelha et al., 2009; Leulmi et al., 2014). Thus far, small mammals have been the focus of SFG investigations in the Americas and Europe (e.g. Rehácek et al., 1985; Barandika et al., 2007; Pacheco et al., 2007; Adjemian et al., 2008; Spitalská et al., 2008; Labruna, 2009; Milagres et al., 2010; Schex et al., 2011; Dantas-Torres et al., 2012; Sosa-Gutiérrez et al., 2014; Obiegala et al., 2017; Fischer et al., 2017).

However, data on rickettsiae in small mammals in southern Africa are lacking. Here we present the first comprehensive study on the prevalence, diversity as well as host and geographic distribution of rickettsiae in small mammals collected at different localities distributed over South Africa and Namibia.

2. Materials and methods

The study is a retrospective study that makes use of rodents and insectivores that were trapped as part of an ongoing parasite ecology research program of Sonja Matthee. Initial studies focused on the rodent genus *Rhabdomys* and localities in the Western Cape Province and as such the genus and province is better represented than others. Parasites were used for various projects and therefore were not available for inclusion in the present study.

From 2004–2012, 1616 small mammals were trapped at two localities in Namibia and 36 in South Africa using Sherman-type live traps. Trapping was mainly conducted during the warmer spring-summer months and in pristine natural vegetation and agricultural areas. At each locality and in each year the same trapping procedure was followed. Line transects were used and traps were placed 10 m apart from one another. The number of traps used per locality ranged from 100 to 200. The aim was to only trap adult individuals of each small mammal species. These were collected for general parasite studies and in order to keep data comparable the confounding factor of age