Coronaviruses in South African Bats

Marike Geldenhuys,¹ Jacqueline Weyer,^{1,2} Louis H. Nel,¹ and Wanda Markotter¹

Abstract

Recent studies in several African countries have provided the first evidence for the presence of coronaviruses in African bats. Here we describe, for the first time, the detection of RNA of 3 unique coronavirus species in the tissues of South African bats.

Key Words: Coronavirus—Alphacoronavirus—South Africa—Bat.

Introduction

The severe acute respiratory syndrome (SARS) epidemic in China in 2002, resulted in 8422 laboratory confirmed human cases with 916 mortalities (Chan-Yeung and Xu 2003). The causative agent was a newly emergent coronavirus (CoV) that may have originated from a similar virus, SARS-related CoV, discovered in the Chinese horseshoe bats (Rhinolophus spp.) (Lau et al. 2005). This stimulated further investigations into the presence and diversity of bat coronaviruses (BtCoV) globally, and these studies have concomitantly led to the description of various novel BtCoVs (Poon et al. 2005, Drexler et al. 2010, Reusken et al. 2010). Studies of African bat species have revealed the presence of bat coronaviruses; unique alphacoronaviruses related to the human coronavirus 229E as well as Betacoronavirus members of the SARS-Rh-BtCoV-related species cluster have been identified (Tong et al. 2009, Pfefferle et al. 2009, Quan et al. 2010). The only evidence for the presence of BtCoV in South Africa is based on a serological survey performed in 2007 that revealed antibodies reactive against SARS-CoV from 2 African bat species, Rousettus aegyptiacus and Mops condylurus, with a seroprevalence of 10.08% (Müller et al. 2007).

In this study, archival samples collected since 2005 from various South African provinces were analyzed for the presence of bat CoVs and compared to previously detected bat CoVs identified in other bat species.

Materials and Methods

Archival fecal material and rectal specimens (tissue and swabs) representing 14 different bat genera collected in South Africa from 2005 onward were tested (Table 1). RNA was extraction with TRIzol (Invitrogen) according to the manufacturer's instructions and screened with a genus-specific double heminested RT-PCR assay after designing alpha- and betacoronavirus–specific primers (originally based on primers from Woo et al. 2005) targeting the RNA-dependent RNA polymerase (RdRp) gene (available on request). PCR amplicons were purified with the Wizard SV gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's instructions and sequenced with the BigDye terminator V3.1 Kit (Applied Biosystems, USA), edited in CLCBio main workbench v6 (CLCbio, Denmark) and aligned using ClustalX in BioEdit (Hall 1999). A neighbor-joining phylogenetic tree was constructed with 1000 bootstrap replicates in MEGA 5 (Tamura et al. 2011).

Results and Discussion

A total of 113 gastrointestinal specimens were analyzed from which 3 specimens tested positively for CoV RNA; *Neoromicia capensis* (Taung, North West), *Miniopterus* spp. (Irene, Gauteng), and *Mops midas* (Makhado, Limpopo). A phylogenetic tree was constructed with 277-bp fragments including all relevant human, animal, and bat coronavirus RdRp sequences (Fig. 1).

On the basis of this partial sequence data, the South African BtCoV sequences clustered within the *Alphacoronavirus* genus. *Miniopterus*-BtCoV Irene/SA/09 (*Mi*-BtCoVIrene) shows clustering with several alphacoronaviruses previously detected within the same host genus, and shares 86.1% and 94.3% nucleotide and amino acid identity, respectively, with *Mi*-BtCoV1A from China (Poon et al. 2005) as well as 88.4% and 97.8% nucleotide and amino acid identity, respectively, with *Mi*-BtCoVKY66 from Kenya (Tong et al. 2009). The majority of the *Miniopterus* BtCoV seems to cluster together, irrespective of the country of origin. This may indicate the existence of an original *Miniopterus* BtCoV ancestor present in

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Bat species	Positive for CoV/ total sampled	Sampling Location(s) in South African provinces
Entesicus hottentotus	0/2	Taung, northwest
Epomophorus wahlbergi	0/11	Amanzimtoti and Durban
	•, ==	Rocktail Bay, St. Lucia.
		Woodhurst, Chatsworth all in KwaZulu Natal
Epomophorus gambianus	0/1	Pafuri, Kruger National Park, Limpopo
Glauconucteris variegata	0/1	Rocktail Bay, St. Lucia, KZN
Hipposideros caffer	0/2	Pafuri. Kruger National Park, Limpopo
Miniopterus natalensis	1/14	Irene Caves, Gauteng
		Peppercorn cave in Modimole, Limpopo
		Venterskroon in Thabela thabeng, northwest
Mops condylurus	0/2	Palaborwa, Limpopo.
Mops midas	1/2	Makhado, Limpopo
Chaerephon spp. ^a	0/1	Pafuri. Kruger National Park, Limpopo
Chaerephon pumilus	0/3	Rocktail Bay, St. Lucia, KZN
Tadarida aegyptiaca	0/4	Taung, North West
Molossidae bats*	0/3	Pafuri. Kruger National Park, Limpopo
		Kgaswane, North West.
Neoromicia capensis	1/10	Taung, North West
		Pafuri. Kruger National Park, Limpopo
		Free Me rehab, Gauteng
Neuromicia nana	0/7	Pafuri. Kruger National Park, Limpopo
Neuromicia helios	0/2	Pafuri. Kruger National Park, Limpopo
Neoromicia zuluensis	0/1	Pafuri. Kruger National Park, Limpopo
Neoromicia spp. ^a	0/2	Hennopspruit, Gauteng
Nycticeinops schlieffenii	0/7	Pafuri. Kruger National Park, Limpopo
Nycteris thebaica	0/2	Rocktail Bay, St. Lucia, KZN
Rhinolophus denti	0/5	Taung, northwest
Rhinolophus capensis	0/1	Labuschagne farm, Louis Trichard, Limpopo
Rhinolophus darlingi damarensis	0/1	Taung, northwest
Rhinolophus landeri	0/1	Pafuri. Kruger National Park, Limpopo
Rhinolophus spp."	0/2	Makhado, Limpopo
Rousettus aegyptiacus	0/3	Pafuri. Kruger National Park, Limpopo
Scotopniius ainganii	0/9	Pafuri. Kruger National Park, Limpopo
Castaulilus lauraastau	0/2	Kgaswane, northwest
Scotophilus leucoguster	0/2	Paruri. Kruger National Park, Limpopo
Scotophilus oiriuis	0/1	Paruri. Kruger National Park, Limpopo
Scotophilus spp.	0/7	Vasuurana northwest
		Reastrail Box St. Lucia K7N
Insectivorous bat species ^a	0/4	Taung and Kgaswane, northwest
nisecuvorous bat species	0/4	Pafuri Kruger National Park Limpone
Total:	3/113	i aturi. Kruget ivational i atk, Emipopo
1 (will	0/110	

TABLE 1. THE NUMBER OF BAT SPECIES ANALYZED FROM VARIOUS LOCATIONS IN SOUTH AFRICA

^aBats not identified to species level.

ancient *Miniopterus* spp. lineages before species divergence and migration to different continents.

No study has previously identified coronaviruses within the *Neoromicia* host species and as such the closest phylogenetic relatives of *Neo*-BtCoV167/SA/07 based on limited RdRp sequences are alphacoronaviruses identified in the *Nyctalus* genus from The Netherlands and Bulgaria. The *Alphacoronavirus* BtCoV/*N. noc*/NL-VM176/10 identified in The Netherlands (Reusken et al. 2010) shares 83.9% nucleotide and 93.2% amino acid identity to *Neo*-BtCoV167/SA/07. BtCoV/*N. lei*/BNM98-30/08 identified in Bulgaria (Drexler et al. 2010) share 84.3% nucleotide and 93.2% amino acid identity to *Neo*-BtCoV167/SA/07.

Müller et al. (2007) identified antibodies against the SARS-CoV in 2 bat species in South Africa, 1 being *Mops condylurus* (Müller et al. 2007). However, no coronavirus RNA has previously been identified within this host genus. This study has detected *Alphacoronavirus* RNA, *Mops*-BtCoV1364/SA/11, in the fecal swab of a dead bat collected from an urban colony of *M. midas* in Limpopo Province. *Mops*-BtCoV1364/SA/11 is closely related to *Chaerephon*-BtKY22 from Kenya (Tong et al. 2009) and shares a 98.5% nucleotide and 100% amino acid identity. Along with the high bootstrap support, it may be concluded that these 2 alphacoronaviruses are different strains of the same virus species first identified from a *Chaerephon*, are very closely related and share an overlapping geographical distribution. Thus, it is possible for interspecies transmissions of this alphacoronavirus to occur between these 2 host genera.



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FIG. 1. Neighbor-joining phylogenetic tree constructed with 277-bp sequence of the RNA-dependent RNA polymerase gene using 1000-bp repetitions. Bootstrap values of less than 40 have been omitted from the tree. Relevant sequences of related coronaviruses are included and the country of origin are indicated by symbols: China (\bullet), Netherlands (\blacksquare), China (\blacktriangle), Bulgaria (\diamond), and United States of America (\triangleright). Coronaviruses detected in this study are indicated in bold. Accession numbers are indicated in square brackets. CoV, Coronavirus.

Conclusions

This study has provided the first identification of bat CoVs RNA present in South African bat species. Three novel bat alphacoronaviruses have been detected in 3 separate provinces with the identification of 2 new bat CoV host species. This may indicate a widespread presence of coronaviruses in Southern African insectivorous bats, as the host ranges of the bat species found to be harboring bat CoVs in this study are not limited to only South Africa.

Mi-BtCoVIrene/SA/09 shows a relation to BtCoV from members of the same host genus, as is often seen with *Mini*-

opterus BtCoV (Poon et al. 2005). However, in the case of *Neo*-BtCoV167/SA/07 and *Mops*-BtCoV1364/SA/11, there are no comparable viruses that have previously been identified from these host species, and as such their closest relatives are derived from different host species. Generally, the trend that has been observed with bat CoVs is host genus/species specificity, with a few documented exceptions (Tong et al. 2009). It is possible that *Mops*-BtCoV1364/SA/11 may be another exception to the trend because another strain of the virus, BtKY22 was identified in Kenya from the *Chaerephon* genus, another member of the Molossinae subfamily. However, evolutionary conclusions surmised based on small sequences

of the RdRp gene may only serve as a preliminary indicator as to the origins and phylogeny of bat CoVs in relation to human CoV and other mammalian CoV.

Bats have been suggested as the gene pools for all mammalian coronavirus lineages (the alpha- and betacoronaviruses), due to the detection of a diverse range of bat coronaviruses that have been found to be similar to the majority of mammalian coronaviruses (Woo et al. 2009). The 2002 SARS epidemic in China is an excellent example of how possible, under conducive circumstances, it is for coronaviruses to spread to intermediate animal species and through such animals come into contact with humans, allowing for the viruses to adapt to human hosts. However, the frequency of such host species transmissions is unknown. Further study of bat CoV within their natural reservoir species becomes necessary to elucidate the global epidemiological understanding of these viruses.

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Author Disclosure Statement

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