Arenaviruses are feared as agents that cause viral hemorrhagic fevers. We report the identification, isolation, and genetic characterization of 2 novel arenaviruses from Namaqua rock mice in Namibia. These findings extend knowledge of the distribution and diversity of arenaviruses in Africa.

Arenaviruses are known to cause severe hemorrhagic fevers across the globe with case fatality rates up to 30% (1). The viruses possess a bisegmented, single-stranded RNA genome with ambisense coding strategy consisting of a small segment coding for the nucleoprotein and glycoprotein and a large (L) segment coding for the RNA-dependent RNA polymerase and matrix protein.

In Africa, Lassa virus (LASV) and Lujo virus are the only known members of the family Arenaviridae that cause human disease (2,3); however, evidence for lymphocytic choriomeningitis virus, another Arenaviridae sp., was recently reported in Gabon (4). Several other arenaviruses of unknown pathogenic potential have also been found in Africa: Gbagroube, Kodoko, and Menekre viruses from western Africa (5,6); Ippy (IPPYV) and Mobala viruses from central Africa; Mopeia, Morogoro, Luna, and Lunk viruses from eastern Africa; and Merino Walk virus (MWV) from southern Africa (7,8). All of these viruses are carried by rodents of the family Muridae.

Until now, no molecular detection of arenaviruses has been reported from Namibia. A study in 1991 described a low seroprevalence (0.8%) for LASV antibodies in humans in northern Namibia (9). Because of lack of data about arenavirus occurrence and effects in southwestern Africa, we conducted a study of small mammals from Namibia to detect infection with arenaviruses.

The Study

During 2010–2012, animal trapping was performed in 8 areas in central and northern Namibia (Figure 1), and samples from 812 rodents and shrews were obtained (Table 1). The animals were dissected in the field and stored individually in a field freezer at –20°C and later at –80°C. For primary arenavirus screening, lung sections of all animals were homogenized, and RNA was extracted and reversely transcribed by using random hexamer primers. Screening was performed by arenavirus genus-specific reverse transcription PCR (RT-PCR) (10) to detect the L genomic segment. From samples testing positive by

Figure 1. Screening for arenaviruses in Namibia. Trapping locations (named according to the nearest urban settlement) of small mammals. Sites where samples positive for new arenaviruses were found are marked by a crossed circle and underlined locality names. Geographic positioning system coordinates of the trapping sites: Ben Hur, 22°87.26′S, 19°21.10′E; Cheetah Conservation Fund (CCF), 16°39.0′E, 20°28.12′S; Mariental, 24°62.08′S, 17°95.93′E; Okahandja, (21°98.33′S, 16°91.32′E); Palmwag, 19°53.23′S, 13°56.35′E; Rundu, 17°56.64′S, 20°05.109′E; Talismanis, 21°84.30′S, 20°73.91′E; Windhoek, 22°49.93′S, 17°34.76′E.
Table 1. Small mammals captured in Namibia during 2010–2012 and tested for arenaviruses*

| Mammal species                  | Common name              | Locality of capture† | No. positive/no. tested |
|---------------------------------|--------------------------|----------------------|-------------------------|
| Aethomys chrysophilus           | Red veld rat             | Be, CCF, Ok, Pa, Ta  | 0/64                    |
| Micaelamyss namaquisensis       |Namaqua rock mouse        | CCF, Ma, Ok, Pa, Ru  | 4/266                   |
| Crocidura fuscomurina           | Bicolored musk shrew     | CCF, Pa, Ru          | 0/4                     |
| Crocidura hirta                 | Lesser red musk shrew    | Ma                    | 0/5                     |
| Dendromys melanos              | Gray climbing mouse      | Ta                    | 0/1                     |
| Elephantulus inufl             | Bushveld sengi           | CCF, Ma, Ok          | 0/14                    |
| Gerbilliscus spp.              | Gerbil                   | Wi                    | 0/6                     |
| Gerbilliscus leucogaster       | Bushveld gerbil          | Be, CCF, Ma, Ok, Pa, Ru, Ta | 0/228                  |
| Gerbillurus paeba              | Hairy-footed gerbil      | Be                    | 0/3                     |
| Gerbillurus setzani            | Namib brush-tailed gerbil| Be                    | 0/1                     |
| Lemniscomys rosalia            | Single-striped grass mouse| Be                    | 0/2                     |
| Mastomys spp.                  | Multimammate mouse       | Be, CCF, Ma, Ok, Pa, Ru, Ta | 0/114                  |
| Mus indutus                    | Desert pygmy mouse       | Ma, Pa                | 0/5                     |
| Petromyseus collinus           | Pygmy rock mouse         | Pa                    | 0/3                     |
| Rhabdomyus pumilio             | Four-striped grass mouse | CCF, Ma, Pa, Ok, Wi  | 0/73                    |
| Saccostomus campestris          | Pouched mouse            | Be, CCF, Ok, Pa, Ru  | 0/17                    |
| Thallomys paedi                 | Acacia rat               | Pa                    | 0/4                     |
| u. u. Soricidae                | Shrew                    | Wi                    | 0/2                     |
| Total                           |                          |                      | 4/812                   |

* Morphologic species identification of the arenavirus positive rodent samples was confirmed by sequencing of partial mitochondrial cytochrome b gene (GenBank accession nos.: N27, KP752173; N73, KP752175; N80, KP752176; N85, KP752174).
† Abbreviations and sampling dates for trapping localities: Be, Ben Hur (1/11/2011); CCF, Cheetah Conservation Fund (02/2011); Ok, Okahandja (06/2012), Pa, Palmwag (09/2010), Ta, Talismanis (12/2011), Ma, Mariental (06/2012), Ru, Rundu (01/2011), Wi, Windhoek (09/2010 and 01/2012).

arenavirus PCR, frozen lung tissue aliquots were homogenized and added to confluent Vero-E6 cells (ATCC CRL-1586; American Type Culture Collection, Manassas, VA, USA) for virus isolation.

For genome sequencing, pellets from ultracentrifuged supernatant of infected cell cultures were lysed, and total RNA was purified. RNA was then subjected to random-primed RT-PCR as described (11). Next-generation sequencing was performed by using a 454 Genome Sequencer Junior (Roche, Indianapolis, IN, USA), and results were aligned against the virus database by using blastn and blastx algorithms (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequencing results matching arenavirus sequences were blasted (Figure 2, panel A). Cell culture isolation was performed with samples N27 and N73 and resulted in 2 novel arenavirus isolates: Mariental virus (MRTV) and Okahandja virus (OKAV), respectively.

The genomes of the 2 arenaviruses were investigated by using next-generation sequencing and RT-PCR Sanger sequencing. The genome data obtained for MRTV and OKAV showed a typical arenavirus nucleotide composition with the L segment (MRTV: 6,840 nt; OKAV: 7,170 nt) coding for RNA polymerase and matrix protein and the S segment (MRTV: 3,360 nt; OKAV: 3,379 nt) coding for glycoprotein and nucleocapsid protein. Table 2 shows the nucleotide and amino acid sequence identities of nucleocapsid and glycoprotein open reading frame with other Old World (i.e., Eastern Hemisphere locations such as Europe, Asia, Africa) representatives of genus Arenavirus. On the basis of the nucleocapsid amino acid identity, OKAV is most related to MWV (75.7% identity). Furthermore, MRTV has the highest amino acid identity with IPPYV (71.4% identity) and with Gbagroube, Lassa, Luna, and Mobala viruses (~70% identity).

In the nucleocapsid-based phylogenetic tree, OKAV clusters with 100% bootstrap support with MWV detected in Myomys unisulcatus rodents in South Africa (Figure 2, panel B), and MRTV forms a clade with IPPYV isolated from Praomys spp. in the Central African Republic. The bootstrap support of this monophyletic group of the tree lies at 56%. The analysis of the glycoprotein open reading frame (Figure 2, panel C) leads to a similar result; OKAV shares the most recent common ancestor with MWV, and MRTV clusters with IPPYV but with a weaker bootstrap support.
We detected and isolated 2 novel arenaviruses in Namibia, OKAV and MRTV. OKAV clearly clustered in relationship with the MWV from southern Africa, but MRTV is a more divergent member of the Old World arenavirus clade. According to amino acid identity and phylogenetic analysis, MRTV was most closely related to IPPYV from the Central African Republic; however, the low bootstrap support precluded a stringent estimation of this closest relative.

These new strains comply with the arenavirus species definition (14) on the basis of amino acid differences in

Table 2. Nucleotide and amino acid identities of Mariental (MRTV) and Okahandja (OKAV) viruses compared with Old World representatives of the genus Arenavirus*

| Virus species       | S segment GenBank accession no. | GPC nt | GPC aa | NP nt | NP aa |
|---------------------|---------------------------------|--------|--------|-------|-------|
| MRTV (N27)†         | KM272987                        | 64.6   | 68.9   | 64.9  | 66.1  |
| OKAV (N73)‡         | GU830848                        | 66.7   | 73.6   | 64.1  | 69.9  |
| Gbagroube           | NC_007905                       | 66.4   | 73.7   | 64.1  | **71.4** |
| Lassa               | NC_007905                       | 67.4   | 73.2   | 65.5  | 69.8  |
| LCMV                | AB261991                        | 57.4   | 57.2   | 61.1  | 63.6  |
| Lujo                | JX017360                        | 47.7   | 38.2   | 60.1  | 56.7  |
| Luna                | AB586646                        | 66.4   | 73.2   | 64.2  | 69.5  |
| Lunx                | NC_018710                       | 57.4   | 54.1   | 61.2  | 62.5  |
| Menekre             | GU830862                        | 66.5   | 72.3   | 65.1  | 68.3  |
| Merino walk         | GU078660                        | 63.8   | 70.2   | 64.9  | 67.3  |
| Mobala              | NC_007903                       | 63.8   | 72.1   | 64.6  | 70.5  |
| OKAV (N73)‡         | KM272988                        | 62.0   | 66.3   | 61.1  | 65.7  |
| Gbagroube           | GU830848                        | 62.9   | 69.4   | 62.3  | 66.2  |
| Lassa               | NC_007905                       | 64.6   | 68.2   | 60.8  | 65.9  |
| LCMV                | AB261991                        | 58.9   | 57.1   | 62.0  | 63.6  |
| Lujo                | JX017360                        | 47.6   | 38.4   | 60.0  | 57.8  |
| Luna                | AB586646                        | 62.5   | 67.1   | 63.0  | 67.2  |
| Lunx                | NC_018710                       | 56.1   | 55.1   | 60.6  | 62.6  |
| Menekre             | GU830862                        | 63.7   | 70.4   | 62.9  | 65.9  |
| Merino walk         | GU078660                        | 64.7   | **76.1** | 68.2  | 75.7  |
| Mobala              | NC_007903                       | 62.0   | 66.5   | 63.6  | 64.5  |

*Identity values are shown for glycoprotein and nucleocapsid open reading frames. Highest aa identity values are shown in **boldface**.

S, small; GPC, glycoprotein; NP, nucleocapsid protein; nt, nucleotide; aa, amino acid; LCMV, lymphocytic choriomeningitis virus; L, large segment.

†Genome composition of MRTV (N27): (Z: 69–371; RdRP: 6,820–473; GPC: 49–1,527; NP: 3,297–1,710). Accession numbers for MRTV (N27) virus sequences: L, KP867841; S, KM272987.

‡Genome composition of OKAV (N73): Z: 56–336; RdRP: 7,121–435; GPC: 51–1,553; NP: 3,315–1,627. Accession numbers for virus sequences for OKAV N73: L, KP867642; S, KM272988; for OKAV N80: L, KM234277; for OKAV N85: L, KM234278.
nucleocapsid of \( \geq 12\% > 20\% \) for both viruses), presence of specific host species, and existence of laboratory isolates. These properties indicate that MRTV and OKAV represent distinct arenavirus species.

These 2 viruses were found in the same host species located within a radius of 300 km. MRTV was found in only 1 sample (of 266); OKAV was detected in samples from 3 animals. Although more unlikely for OKAV than for MRTV, the possibility of a spillover infection to *M. namaquensis* from a still unknown reservoir host cannot be ruled out for either virus.

The Namaqua rock mouse’s habitat includes the tree and shrub savannahs of Namibia and most parts of southern Africa, including Namibia, South Africa, Botswana, Zimbabwe, and parts of Mozambique. These locations imply the possible occurrence of MRTV or OKAV in larger regions of the continent. Cell culture isolates and genomic sequence data are the first prerequisites for evaluating the public health relevance of these new viruses. Our findings extend the knowledge of geographic distribution and genetic diversity of arenaviruses in Africa.

**Acknowledgments**

We thank C. Chimimba for advice in small mammal systematics, P. Chimwanurombe for advice during preliminary screening for arenaviruses, and C. Priemer for technical support.

Trapping in Namibia was carried out under research permit nos. 1572/2011, 1666/2012 and 1794/2013, granted by Namibia’s Ministry of Environment and Tourism.

This study was supported by Deutsche Forschungsgemeinschaft (grant KR1293/13-1) and by the Slovak Research and Development Agency under the contract no. DO7RP-0008-09.

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