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Serological and molecular characterization of leptospira serovar Kenya from captive African giant pouched rats (*Cricetomys gambianus*) from Morogoro Tanzania

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KEYWORDS

Leptospira • Tanzania • Characterization • *Cricetomys* • Rats • Serovar

Abstract



Two identical leptospiral isolates coded Sh9 and Sh25 obtained from the urine of captive African giant pouched rats (*Cricetomys gambianus*), destined for use as biodetector of antipersonnel landmines were typed as serovar Kenya using cross-agglutination absorption test and DNA fingerprinting with the insertion element sequences IS1533 and IS1500 derived primers. The two isolates were previously characterized using cultural and serological – microagglutination test as pathogenic leptospires of the serogroup Ballum, closely related to serovars Kenya and Peru. To our knowledge, this is the first reported in-depth characterization of leptospira isolates from Tanzania.

Received 2 February 2004, Accepted 9 February 2004

DIGITAL OBJECT IDENTIFIER (DOI)

10.1016/j.femsim.2004.02.002 [About DOI](#)

1 Introduction



Leptospirosis is an important zoonosis caused by a spirochete, *Leptospira interrogans*"sensu lato". Initially described in Germany by Weil (1886), leptospirosis is worldwide distributed, especially in the tropical and subtropical countries where sanitation facilities are poor and survival of pathogenic leptospire in the environment is optimal [1]. Any mammal can be infected with one or more leptospira serovars, however, rodents are the most commonly affected animals, which are also the major natural reservoirs of this microorganism [2-4]. In the tropics, peridomestic and field rodents such as *Rattus rattus* (L., 1758), *Mastomys natalensis* (Smith 1874) and *Cricetomys gambianus* (Waterhouse, 1840) are amongst the primary reservoirs of leptospire [3,5].

In humans, leptospirosis is a disease with a variety of clinical manifestations, from mild, flue-like symptoms to severe, potentially fatal septicaemic complications. Due to its protean character, leptospirosis mimics many other diseases and hence difficult to diagnose clinically. The support of a specialized laboratory to establish the diagnosis is therefore indispensable [6].

The microagglutination test (MAT) is the standard assay in serodiagnosis of leptospirosis [7]. Other laboratory methods include: enzyme-linked immunosorbant assay (ELISA) [8], indirect fluorescent antibody test (IFAT) and, more recently, polymerase chain reactions (PCRs) [9-12].

Humans usually get infected after coming into contact with urine of infected rodents. Intraspecific and interspecific transmission within susceptible hosts may persist in an environment that has been contaminated with urine for a long time, especially if favorable climatic conditions such as warm, humid environment exist [2,5].

In the East and Central African region, leptospirosis has been reported since three decades [13-17]. In any event, leptospirosis is predominantly an occupational disease which affects persons who come into frequent contact with rodents or other infected animals. The most frequently affected occupational groups include: agricultural workers, cattle farmers, and sewage workers. Infection is further facilitated by conditions that facilitate the penetration of the leptospire through mucosa or openings (injuries) of the skin [18-20]. Persons handling wild rodents as pets, food, or for research activities may therefore be at high risk of infection [5].

In Tanzania there is only a limited knowledge on the prevalence of leptospirosis in animals and humans [2]. Nevertheless, the diverse epidemiological factors which predispose the existence of leptospirosis in Tanzania are prevalent. These include warm, humid/swampy environments and the presence of commensal rodents, which are the natural reservoirs of leptospire. In addition, clinical pyrexia of unknown origin (PUO) cases are common among vulnerable occupation groups which could be easily misdiagnosed to other diseases common in this country, such as malaria, dengue, or typhoid. There is therefore a need for broader epidemiological and clinical research on this pathogen.

In recent studies involving the training of the African giant pouched rats (*C. gambianus*) to detect landmine explosive traces by smell, the urine of newly captured rats was screened for *Leptospira* species. The aim of screening was to determine to what extent persons handling (breeding and training) these rats might be at risk of infection with pathogenic leptospire. Preliminary findings have demonstrated the presence of leptospire belonging to serogroup Ballum in the urine of the *Cricetomys* rats [5].

This paper reports on further serological and molecular characterization of Tanzania's leptospire isolates from the African giant pouched rats (*C. gambianus*).

2 Materials and methods



Two leptospira isolates (Sh9 and Sh25) from African giant pouched rats, preliminarily characterized by Machang'u et al. [5] were further characterized serologically by cross-agglutination absorption test (CAAT) and molecular techniques (PCR).

2.1 Serotyping

2.1.1 Cross-agglutination absorption test

The CAAT was performed as described by Dikken and Kmety [21] based on recommended criteria for leptospira serovar determination [22].

Serovars for CAAT were selected from the Ballum serogroup by establishing homologous MAT titres with six serovar specific antisera raised in rabbits. These included serovars: Arborea, Ballum, Guangdong, Castellonis, Kenya and Peru. Heterologous MAT titres were established using reference rabbit antisera for serovars of the Ballum group against the isolates as described by Hartskeerl et al. [23].

2.2 Molecular typing by polymerase chain reactions

2.2.1 DNA extraction

DNA was isolated using the Anansa[®] Fast 'n' Easy Genomic DNA Purification kit (Tebu-bio laboratories, Cedex, France). The concentration of DNA was estimated visually against a 100–1000 bp DNA standard (smart ladder[®], Eurogentec, Belgium) in a 1.5% agarose gel electrophoresis, stained with ethidium bromide.

2.3 Pathogenic status

The pathogenic status of Sh9 and Sh25 isolates was further investigated by PCR with primer sets, which differentiate between saprophytic and pathogenic leptospires as described elsewhere [11,24,25].

One set consisted of two primer pairs: Sapro 1 (5'-AGA-AAT-TTG-TGC-TAA-TAC-CGA-ATG-T-3') and Sapro 2 (5'-GGC-GTC-GCT-GCT-TCA-GGC-TTT-CG-3') which specifically amplifies saprophytic leptospiral DNA; and primer pair Lepat 1 (5'-GAG-TCT-GGG-ATA-ACT-TT-3') and Lepat 2 (5'-TCA-CAT-CG(CT)TGC-TTA-TTT-T-3') which amplifies pathogenic leptospiral DNA [24].

The other set consisted of primer pairs LU (5'-CGG-CGC-GTC-TTA-AAC-ATG-3') and rLP (5'-ACC-ATC-ATC-ACA-TYG-CTG-C-3'), and LP1 (5'-GAT-TTT-TCG-GGT-AAA-GAT-TCA-TT-3') and a 1190 (5'-TTG-CCC-TAG-ACA-TAA-AGG-CCA-3') for detection of pathogenic leptospires [25].

The isolates were further subjected to PCR amplification using other primer pairs designed for the detection of pathogenic leptospires, namely: G1 (5'-CTG-AAT-CGC-TGT-ATA-AAA-GT-3') and G2 (5'-GGA-AAA-CAA-ATG-GTC-GGA-AG-3'); B64I (5'-ACT-AAC-TGA-GAA-ACT-TCT-AC-3') and B64II (5'-TCC-TTA-AGT-CGA-ACC-TAT-GA-3') as described by Gravekamp et al. [11].

2.4 DNA fingerprinting

DNA fingerprinting was done by PCR amplification using primers derived from IS1533 and IS1500 insertion element sequences as described by Zuerner and Bolin [9,10].

The insertion element sequence IS1533 derived primers were: EPR-2, 5'-CTC-GCA-TCT-AAC-CCA-CGT-TT-3' and EPL-2, 5'-AGA-TTT-ACT-GCT-CCG-GAT-GG-3', while the primers from IS1500 insertion element used were iP1, 5'-CGT-TAG-CCA-TGC-TTT-GAA-TCG-AA-3' and iM16, 5'-CGC-AGT-CGC-TGA-GTC-CTT-CTT-T-3'. The insertion element sequence derived primers were used either singly or as primer pair combinations. All primers used were obtained from Eurogentec[®] Company (Belgium).

3 Results



3.1 Serotyping

3.1.1 Cross-agglutination test

In the cross-agglutination test, isolates Sh9 and Sh25 gave agglutination titres greater than 10% with serovars Kenya, Peru and Ballum, whereas less than 10% or no agglutination was found with serovars Arborea, Guangdong or Castellonis (Table 1).

Table 1 Homologous and heterologous (cross-agglutination) MAT comparative titres of isolates Sh9, Sh25 with rabbit antisera for reference serovars of serogroup Ballum

Serogroup Ballum

Rabbit antiserum against serovar:	Homologous titre with reference serovars	Heterologous titre with isolates Sh25 ^a	Cross-agglutination (%)
Arborea	1:2560	1:160	6.2
Ballum	1:640	1:320	50
Guangdong	Not done	<1:20	0
Castellonis	Not done	<1:20	0
Kenya	1:5120	1:2560	50
Peru	1:1280	1:320	25

^aResults of Sh9 isolate (not shown) were similar to those of Sh25 isolate.

3.1.2 Cross-agglutination absorption test

Results of the cross-agglutination absorption test between serovars Kenya, Peru, Ballum and isolates Sh9 and Sh25 with the percentages of antibody titres remaining after cross-agglutination absorption were as shown in Table 2.

Table 2 Cross-agglutination absorption test (CAAT) between reference serovars Kenya, Peru, Ballum, isolates Sh9 and Sh25

Antiserum against	Absorbing strain	Homologous titre before absorption	Homologous titre after absorption	Remaining titre (%)
Sh25 isolate ^a	Kenya	10,240	640	6.2
Kenya	Sh25	10,240	320	3.1
Sh25 isolate	Peru	10,240	5120	50
Peru	Sh25	5120	Not done	
Sh25 isolate	Ballum	10,240	10,240	100
Ballum	Sh25	640	Not done	

^aResults of Sh9 isolate (not shown) were identical to those of Sh25.

3.2 Molecular characterization**3.2.1 Pathogenic status by PCR**

PCR amplification of the two isolates produced bands with specific primers for amplification of pathogenic leptospiral DNA. PCR products of 330 bp were obtained with Lepat 1 and Lepat 2 primers (Fig. 1), while products of 1008 and 420 bp were obtained with primer pairs LP1 and a 1190, and LU and rLP, respectively. The two isolates were also amplified by G1 and G2 primer pair, whereas no PCR products were obtained with B64I and B64II, and Sapro 1 and Sapro 2 primers.

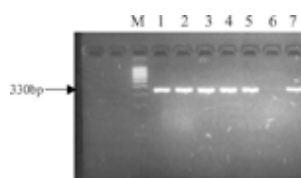


Fig. 1 PCR products (330 bp) of Sh9 and Sh25 and other isolates (not from giant rats), and reference leptospires amplified using Lepat 1 and Lepat 2 primers for detection of pathogenic leptospires. DNA size marker (100–1000 bp) – lane (M), RM1 bovine isolate (lane 1), RM4 bovine isolate (lane 2), RM7 bovine isolate (lane 3), Sh9 isolate (lane 4), Sh25 isolate (lane 5), reference saprophyte – serovar Semarang, strain Veldrat Semarang (lane 6), reference pathogen – serovar Mwoголо, strain

Korea (lane 7). The products were separated by electrophoresis in a 3% agarose gel and stained with ethidium bromide.

[Normal View]

3.2.2 DNA fingerprinting

When subjected to DNA fingerprinting using primers derived from insertion elements sequence IS1533 and IS1500, isolates Sh9 and Sh25, and the reference serovars Kenya and Peru generated the DNA fingerprinting profiles shown in Figs. 2 and 3, respectively.

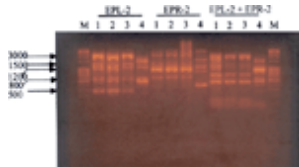


Fig. 2 PCR generated DNA fingerprint of Sh9 and Sh25 isolates compared with reference serovars Kenya and Peru using primers derived from insertion element IS1533. Primers were EPL-2 alone, EPR-2 alone and combination of the two primers (EPL-2 and EPR-2). DNA size marker (100–5000) – (lane M), serovar Kenya (lane 1), Sh9 isolate (lane 2), Sh25 isolate (lane 3) and serovar Peru (lane 4). The fingerprinting products were separated by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

[Normal View]

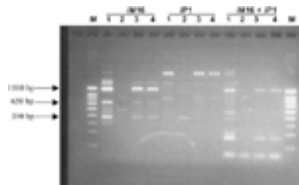


Fig. 3 PCR generated DNA fingerprinting of isolates Sh9 and Sh25 compared with the fingerprint generated from reference serovars Kenya and Peru. PCR fingerprinting was done using primers derived from insertion element IS1500, namely; iM16 and iP1. Primers were used as single primers and as combination of iM16+iP1. DNA size marker (100–1000 bp) – (lane M), serovar Kenya (lane 1), serovar Peru (lane 2), Sh9 isolate (lane 3), Sh25 isolate (lane 4). The fingerprinting products were separated by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

[Normal View]

4 Discussion



This study has shown an overall similarity of the isolates Sh9 and Sh25 with serovar Kenya. On the basis of the cross-agglutination between the isolates and leptospiral serovars of the Ballum group, a close relationship of more than 10% was found with serovars Kenya, Peru and Ballum. Lower (<10%) or absence of cross-agglutination titres (<10%) with the other serovars of the Ballum group (Arborea, Guangdong and Castellonis) suggest that the isolates Sh9 and Sh25 are not closely related to these serovars (Table 1).

Based on the results of the CAAT, it was shown that isolates Sh9 and Sh25 are serologically identical and belong to serovar Kenya of serogroup Ballum, because after cross-absorption with adequate amount of heterologous antigen, less than 10% of the homologous titre remained in the antisera of serovar Kenya, isolates Sh9 and Sh25 (Table 2). However, since more than 10% of the homologous titre remained in the antisera of the isolates after CAAT with serovars Peru and Ballum, it shows that the two isolates could be related but not similar to serovars Peru or Ballum [22].

The novel PCR methods using primer pairs Lepat 1 and Lepat 2 (Fig. 1), LP1 and a 1190, LU and rLP and G1 and G2 (not shown) for pathogenic leptospires, confirmed that the two leptospiral isolates (Sh9 and Sh25) from the African giant pouched rats are pathogenic [11,24,25].

The pathogenicity status of the isolates was further confirmed by failure to produce PCR products with Sapro

1 and Sapro 2 primer pair designed for amplification of saprophytic leptospires (not shown).

Molecular fingerprinting by PCR confirmed that both Sh9 and Sh25 are identical to serovar Kenya of serogroup Ballum. PCR-generated fingerprinting profiles (with IS1533 and IS1500 derived primers) of the two isolates were identical to those of serovar Kenya (Figs. 2 and 3). This similarity was especially evident when IS1533 insertion element derived primers were used in the amplification, suggesting that there are more copies of the IS1533 than IS1500 insertion element in serovar Kenya and the isolates. This is a further indication that isolates Sh9 and Sh25 are identical to serovar Kenya. Based on the results of serological and molecular characterization analyses, it can therefore be concluded that isolates Sh9 and Sh25 belong to serovar Kenya.

Serovar Kenya was first isolated in 1968 from a pouched rat (*Saccostomus campestris*) in neighboring Kenya by Njenga and co-workers [15]. Our conclusion that isolates Sh9 and Sh25 belong to serovar Kenya, thus corroborates with previous findings in the East African region.

Although the definitive prevalence of the leptospires in the *Cricetomys* rats is yet to be established [5], it can be concluded that the *Cricetomys* rats are also carriers of the serovar Kenya in the East African region. Persons handling the rats ought to take appropriate precautionary measures to protect themselves from infection. It is further recommended that *Cricetomys* rats that are newly captured from the wild for breeding or research purposes must be quarantined and screened for leptospires. Where necessary, the rats should be vaccinated against the disease.

Acknowledgements



We thank Ms. Mirjam Engelbert and Marije Liem of the Department of Biomedical Research (KIT, Amsterdam, the Netherlands) for their great assistance with laboratory work and preparation of figures.

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