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New *Leptospira* serovar Sokoine of serogroup Icterohaemorrhagiae from cattle in Tanzania.

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Identification of *Leptospira* isolates from cattle was carried out to establish prevalent *Leptospira* serovars. The prevalence of leptospirosis is generally high in domestic animals and rodents in Tanzania. Serological typing was done based on monoclonal antibodies and standard Cross-Agglutination Absorption Test (CAAT). Molecular typing involved pathogenic and saprophytic-specific PCRs, and a PCR specifically amplifying DNA from species *Leptospira kirschneri*. DNA fingerprinting with primers derived from sequences of insertion elements IS*1500* and IS*1533* was done. Both serological and molecular characterization indicated that one of the *Leptospira* isolates coded RM1, present a new serovar of species *Leptospira kirschneri* of serogroup Icterohaemorrhagiae. The serovar name Sokoine is proposed for this new *Leptospira* isolate.

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Relatively few cases of leptospirosis are recorded on the African continent mainly due to difficulties in diagnosis in both human and animals; hence the disease is not well investigated. However, the prevailing climatic and socio-economic environments are favourable for a high incidence of this disease. Previous findings in Tanzania (Machang'u *et al*. 1997, 2003, 2004) showed that leptospirosis is much more common than generally thought. Currently, twenty *Leptospira* serovars have been described in Africa. Eleven of these serovars belong to species *Leptospira kirschneri* with eight being found in the Democratic Republic of Congo (Zaire), two in Kenya and one in Ghana (Faine *et al.*, 1999). This suggests that *L. kirschneri* may be the prevalent *Leptospira* species, especially in the central and eastern Africa. Areas of high prevalence, such as Tanzania, might be harbouring many serovars, which have not yet been described. Consistent with this assumption, we describe in this paper the isolation and identification of a new *Leptospira kirschneri* serogroup Icterohaemmorhagiae, proposed serovar Sokoine, strain RM1 from cattle in Tanzania.

Leptospira isolates and the known pathogenic and saprophytic strains used in this study are listed in Table 1 below. Serovar Copenhageni, strain Wijnberg was used as the reference pathogen, whereas serovars Semaranga and Patoc served as typical saprophytic reference strains. The EMJH and Fletcher culture media were used to isolate and grow the leptospires (Faine, 1982). Leptospires were isolated from cows from the slaughterhouse of city of Morogoro, Tanzania. Urinary bladders were aseptically punctured to obtain 0.5 ml aliquots of urine, which were then inoculated into 5 ml of Fletcher's medium, supplemented with 5-Fluorouracil (200μg/ml) as a selective inhibitor of contaminating micro-organisms (Faine,

1982). Cultures were incubated at ambient temperature (25-30°C) and examined weekly over a period of 8weeks using dark-field (DF) microscopy (Faine, 1982; Machang'u *et al*., 1997).

The pathogenic status of the isolates was determined by conventional methods and pathogen-specific PCR. The conventional methods consisted of determining *Leptospira* growth rates in EMJH medium at 13°C and in EMJH medium containing 8-azaguanine according to Johnson and Harris (1967), and Johnson and Rogers (1964), respectively.

Briefly, 0.1ml culture of the RM1 isolate was inoculated in 5ml EMJH medium in four tubes and duplicates incubated at 13°C and at 30°C to determine the growth rates at a low and high temperature respectively. 5ml EMJH media with and without 8-azaguanine (225µg/ml) was inoculated in "duplo" with 0.1 ml of RM1 isolate and then incubated at 30°C to determine inhibition (reduction) of growth in the presence of 8-azaguanine. *Leptospira* growth was determined under DF microscopy and by spectrophotometry measuring of culture density at 420nm (Spectrophotometer, DU Series 62, Beckman Instruments, Inc. Fullerton, CA). Pathogenic strain Wijnberg and saprophytic strain Patoc I (Table 1) were included in the test for comparison of growth responses.

DNA was extracted from fully-grown cultures using Anansa® Fast 'n' Easy Genomic DNA purification kit (Tebu-Bio Laboratories, Cedex, France) and the method described by Boom *et al*. (1990). The quantity of extracted DNA was estimated by electrophoresis in 1.5 % agarose stained with ethidium bromide through comparing the intensity of the genomic DNA bands with a standard DNA size marker (100-1000 bp, Smart ladder®, Eurogentec, Belgium). To determine the pathogenic status of the Leptospira isolate a PCR analysis was performed as described by Murgia *et al*. (1997) and Perolat *at al*. (1998). Primer pairs used included Lepat 1 and Lepat 2, specifically amplifying a 330 bp fragment of pathogenic

leptospires; and Sapro 1 and Sapro 2, which specifically generate a 240 bp product from saprophytic leptospires (Murgia *et al*., 1997). Other primer pairs were LP1 and *a*1190 and LU and rLP, which amplify DNA from pathogenic leptospires producing specific amplicons of 1008 bp and 420 bp, respectively (Perolat *et al*., 1998).

The PCR procedure described by Gravekamp *et al*. (1993) was used to determine whether isolate RM1 belonged to species *L. kirschneri*. Primer pairs G1 and G2 that specifically amplify a 285 bp DNA fragment from all pathogenic *Leptospira* species, except *L. kirschneri*, and B64I and B64II specifically amplifying a 563 bp from *L. kirschneri* were used. Reference saprophytic and pathogenic strains were used as controls.

DNA fingerprinting was carried out as described by Zuerner *et al*. (1995, 1997).

Primers derived from insertion element sequences IS*1533* and IS*1500*, consisting of EPL-2 and EPR-2, and iP1 and iM16 respectively, were used separately and in combination. The generated DNA fingerprint of RM1 isolate and other *Leptospira* strains were compared following separation by electrophoresis in a 1.5 % agarose stained with ethidium bromide.

Isolate RM1 was subjected to serological microagglutination with 42 rabbit sera representative for all pathogenic and 4 saprophytic serogroups. The rabbit sera were prepared as described by Faine (1982) and the microscopic agglutination test (MAT) was carried out as described by Cole *et al* (1973). Monoclonal antibody (mAb) typing was done using a panel of mAbs (F12C3-10, F20C3, F20C4-1, F52C1, F52C2, F70C4-1, F70C7-8, F70C13-1, F70C14-6, F70C20-3, F70C24-14, F70C26-1, F82C1-3, F82C2-2, F82C7-3, F82C8-4, F89C3-3, and F89C12-4) that characteristically agglutinate serovars belonging to serogroups Icterohaemorrhagiae and Sarmin as described by Korver *et al*. (1988). Reference serovars of Icterohaemorrhagiae and Sarmin groups (Table 1) were included in this test for comparison of their agglutination patterns with that of RM1 isolate.

Cross agglutination absorption test (CAAT), the gold standard test for serological classification of *Leptospira* serovars was repeatedly carried out by Sokoine University of Agriculture (Tanzania) as described elsewhere (TSCL, 1987; Dikken & Kmety, 1978). The CAAT results were confirmed by the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, Amsterdam, the Netherlands.

Results from this study revealed that RM1 isolate is a pathogenic *Leptospira* as indicated by suppressed growth both at $13^{o}C$ and in the presence of 8-azaguanine. PCR with pathogenic primers Lepat 1 and Lepat 2 gave a DNA product of 330 bp (Fig. 1). No DNA product was generated using the saprophytic *Leptospira* specific primers Sapro 1 and Sapro 2. In addition, a 1008 bp PCR product was obtained with the primers LP1 and *a*1190 (Fig. 2) and a 420 bp DNA product were obtained with primer pair LU and rLP (not shown). These findings support the pathogenic status of RM1. Strain RM1 was PCR positive with *Leptospira kirschneri* – specific primer pair (B64I and B64II) by generating an amplicon of 563 bp, while no product was generated with primers G1 and G2. This suggests that RM1 is a strain of species *L. kirschneri* (Gravekamp *et al*., 1993). PCR-based fingerprinting with iM16 primer derived from IS*1500* produced a pattern from RM1 DNA, which was closely related to that of serovar Ndahambukuje, and somehow related to other *L. kirschneri* and *L. interrogans* strains (not shown). Distinct DNA patterns were obtained from serovar Ndambari and Bogvere that produced few bands for comparison. Apparently, RM1 is genotypically closely related to serovar Ndahambukuje. However, the iM16 based fingerprinting was not informative enough to explain the genetic relatedness of these serovars. The DNA fingerprinting with IS*1533* derived primers (EPL 2 and EPR 2) did not yield DNA profiles with multiple bands from the *L. kirschneri* strains included in the analysis

to enable comparison (not shown). This probably suggests that there are only a few copies of IS*1533* element in these strains.

Sero-agglutination was observed with the sera specific for serogroups Icterohaemorrhagiae, Canicola and Sarmin. Further agglutination tests with rabbit sera against individual serovars of each of the three groups revealed highest agglutination titres with 13 of the 17 serovars of the Icterohaemorrhagiae group, one out of six serovars of Sarmin group and one out of thirteen 13 serovars of the Canicola group. These findings suggested that RM1 most likely belongs to serogroup Icterohaemorrhagiae, or to the serologically closely related serogroup Sarmin.

Subsequent typing with a panel of mAbs, which characteristically agglutinates serovars of the serogroups Icterohaemorrhagiae and Sarmin, revealed the highest similarity with serovars from the Icterohaemorrhagiae group; while relationship with the Sarmin group was virtually ruled out (results not shown). Therefore we conclude that RM1 is a serovar of serogroup Icterohaemorrhagiae. The agglutination profile obtained with RM1 was not identical with any of the established serovars of serogroup Icterohaemorrhagiae. Best fits were found with the histograms of serovars Ndahambukuje and Ndambari (Fig. 3 and Fig. 4). The observations suggest RM1 to be a new serovar of the Icterohaemorrhagiae group.

CAAT was performed repeatedly with serovars of serogroup Icterohaemorrhagiae, of which the rabbit sera showed high cross-agglutination titres (> 10% compared to the homologous titre) with RM1 and *vice versa*. The CAAT results (Table 2) indicated that none of the rabbit sera, after heterologous absorption, gave a remaining titre of less than 10 % in both forward and reversed reactions. According to the definition of the TSCL (1987) this finding further show that RM1 represents a new serovar of serogroup Icterohaemorrhagiae.

This study has shown that RM1 isolate is a new pathogenic serovar of *Leptospira*. It can be classified as a serovar in the serogroup Icterohaemorrhagiae, belonging to species *Leptospira kirschneri*. We propose the name Sokoine for this new serovar. The proposed serovar Sokoine is serologically related to serovars Ndambari and Ndahambukuje both isolated from patients in the Democratic Republic of Congo (Zaire). However, the repeated standard CAAT results indicated that there are marked serological differences that justify the recognition of RM1 strain as a new serovar (TSCL, 1987). Additionally, serovar Sokoine is genotypically closely related to serovar Ndahambukuje although the IS*1500* and IS*1533* fingerprints did not provide enough DNA profiles to conclude on the levels of relationships. It might be hypothesised that the observed serological and genotypic relationship between the *L. kirschneri* serovars Ndahambukuje and Ndambari and the proposed serovar Sokoine from Tanzania, suggest that these serovars evolved from a common ancestor, composing a prevalent group of related serovars in East and Central Africa and possibly have cattle as a natural host. Further studies shall be needed to confirm this hypothesis. These findings corroborate with the *L. kirschneri* prevalence in Africa (Faine *et al*., 1999).

Short description of *Leptospira* serovar Sokoine.

The proposed *Leptospira* serovar Sokoine strain RM1 was isolated from cattle in a slaughterhouse in Morogoro, Tanzania. Serovar Sokoine is deposited under its own serovar and strain name (no accession numbers) in two *Leptospira* culture collection centres: (1) The WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, of the Royal Tropical Institute, Amsterdam, the Netherlands, and (2) the WHO Collaboration Centre for Diagnosis, Reference, Research and Training in Leptospirosis, Port Blair,

Andaman and Nicobar Islands, India. RM1 grows well in *Leptospira* culture media (Fletcher's and EMJH) at ambient temperature. The Growth of this strain is inhibited at 13°C and by 8-azaguanine, which is consistent with a pathogenic status. Genomic DNA of strain RM1 is PCR positive with primers designed for pathogenic leptospires, and specific primers for amplifying DNA from strains of *L. kirschneri*. Serovar Sokoine is distinct from all other recognised serovars of the Icterohaemorrhagiae group on the basis of CAAT results. It gives highest titres in agglutination with antibodies to various serovars of Icterohaemorrhagiae group, and hence can serve as broad antigen for preliminary serological diagnosis of infection with leptospires of Icterohaemorrhagiae group.

This isolate can be classifies as *Leptospira kirschneri* serogroup Icterohaemorrhagiae serovar Sokoine strain RM1. The naming of serovar Sokoine is given in the honour of the Sokoine University of Agriculture in Morogoro, Tanzania, where the research on leptospirosis in Tanzania was pioneered.

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References

1. **Boom, R., Gol, C. J., Salimans, M. M, Jansen, C. L., Wertheim-van Dillen, P.M., & Van der Noordaa, J. (1990).** Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* ***28****, 495.*

2. **Brenner, D. J., Kaufmann, A. F., Sulzer, K. R., Steigerwalt, A. G., Rogers, F. C., & Weyant, R. S. (1999)**. Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. Nov. and four new *Leptospira* genomospecies. *Int J Syst Bacteriol* **49**, 839-858

3. **Cole, J.R., Sulzer, C.R. & Pursell, A.R. (1973)**. Improved microtechnique for the leptospiral agglutination test. *Applied Microbiol* ***25,*** *976 -980.*

4. **Dikken, H. & Kmety, E. (1978).** Serological typing methods of leptospires, p.260-295. *In* Bergan, T. and Norris, R. (ed.), Methods in microbiology, vol. 11. Academic Press, New York.

5. **Faine, S. (1982).** Guidelines for the control of leptospirosis. *WHO offset Publ* ***67***. Geneva.

6. **Faine, S., Adler, B., Bolin, C. & Perolat, P. (1999).** Leptospira and leptospirosis, 2nd edition, Medisci, Melbourne, Australia.

7. **Gravekamp, C., Van de Kemp, H., Franzen, M., Carrington, D., Schoone, G.J., Van Eys, G.J.J.M., Everard, C.O.R., Hartskeerl, R.A. & Terpstra, W.J. (1993).** Detection of seven species of pathogenic leptospires by PCR using two sets of primers. *J Gen Microbiol* ***139****, 1691-1700.*

8. **Johnson, R.C. & Rogers, P. (1964)**. Differentiation of pathogenic and saprophytic leptospires with 8-azaguanine. *J Bacteriol vol* **88 (6),** 1618-1623.

9. **Johnson, R. C. & Harris, V. G. (1967).** Differentiation of pathogenic and saprophytic leptospires. I. Growth at low temperatures. *J Bacteriol* **94,** 27-31

10. **Kmety, E. & Dikken, H. (1993).** Classification of the species *Leptospira interrogans* and history of its serovars, University Press Groningen, Groningen The Netherlands.

11. **Korver, H., Kolk, A.H.J., Vingerhoed, J., Leeuwen, J van. & Terpstra, W.J. (1988).** Clasiffication of the serovars of Icterohaemorrhagiae serogroup by monoclonal antibodies. *Isr J Vet Med* **44-1**, 15-18.

12. **Machang'u, R.S., Mgode, G. & Mpanduji, D. (1997).** Leptospirosis in animals and humans in selected areas of Tanzania. *Belg J Zool Vol* **127,** Suppl1:97 – 104, Brussels.

13. **Machang'u, R., Mgode, G., Asenga, J., Mhamphi, G., Hartskeerl, R., Goris, M., Cox, C., Weetjens, B. & Verhagen, R. (2003).** Characterization of Leptospira isolates from captive giant pouched rats, *Cricetomys gambianus*. In: Rats, Mice and People, Rodent Biology and Management (Singleton, G.R., Hinds, L.A., Krebs, C.J., and Spratt, M.D., Eds), pp 40-42.
Australian Centre for International Agricultural Research, Canberra.

14. **Machang'u, R. S., Mgode, G. F., Assenga, J., Mhamphi, G., Weetjens, B., Cox, C., Verhagen, R., Sondij, S., Goris M.G. & Hartskeerl, R. A. (2004).** Serological and molecular characterization of leptospira serovar Kenya from captive African giant pouched rats (*Cricetomys gambianus*) from Morogoro Tanzania.
FEMS Immun. & Med. Microbiol **41**, 117-121

15. **Murgia, R., Riquelme, N., Baranton, G. & Cinco, M (1997).** Oligonucleotides specific for pathogenic and saprophytic leptospira occuring in water. *FEMS Microbiol Let*. **148,** 27-34.

16. **Perolat, P., Chappel, R.J., Adler, B., Baranton, G., Bulach, D.M., Illinghurst, M.L., Letocart, M., Merien, F. & Serrano, M.S. (1998).** *Leptospira fainei* sp. nov., isolated from pigs in Australia. *Int J Syst Bacteriol* **48,** 851-858.

17. **Subcommittee on the Taxonomy of Leptospira (TSCL) (1987).** *Int J Syst Bacteriol* **37**, 472-473

18. **Zuerner, R. L., Alt, D. & Bolin, C. A. (1995).** IS*1533*-based PCR assay for identification of *Leptospira interrogans* "sensu lato" serovars. *J Clin Microbiol* **33**, 3284-3289.

19. **Zuerner, R. L. & Bolin, C. A. (1997).** Differentiation of *Leptospira interrogans* isolates by IS1500 hybridization and PCR assays. *J Clin Microbiol* **35,** 2612-2617.

Table 1. List of *Leptospira* strains used in this study.

No	Species	Serogroup	Serovar	Strain	Reference.
1	*L. kirschneri*	Icterohaemorrhagiae	Sokoine	RM1-cattle	Described in this paper
2	Unknown	Grippotyphosa	RM4 isolate	RM4 -cattle	Unpublished
3	Unknown	Grippotyphosa	RM7 isolate	RM7- cattle	
4	*L. borgpetersenii*	Ballum	Kenya	Sh9 – giant rats	Machang'u *et al*.,2004
5	*L. borgpetersenii*	Ballum	Kenya	Sh25 - giant rats	
6	*L. biflexa*	Semaranga	Patoc	Patoc I	Brenner *et al*.,1999
7	*L. meyeri*	Semaranga	Semaranga	Veldrat Semarang 173	
8	*L. interrogans*	Icterohaemorrhagiae	Copenhageni	Wijnberg	
9	*L. kirschneri*	Icterohaemorrhagiae	Mwogolo	Mwogolo	
10	*L. kirschneri*	Icterohaemorrhagiae	Ndambari	Ndambari	
11	*L. kirschneri*	Icterohaemorrhagiae	Ndahambukuje	Ndahambukuje	
12	*L. kirschneri*	Icterohaemorrhagiae	Bogvere	LT 60-69	
13	*L. kirschneri*	Icterohaemorrhagiae	Dakota	Grand River	
14	*L. interrogans*	Icterohaemorrhagiae	Icterohaemorrh agiae	RGA	
15	*L. interrogans*	Icterohaemorrhagiae	Copenhageni	M20	
16	*L. interrogans*	Icterohaemorrhagiae	Lai	Lai	
17	*L. interrogans*	Icterohaemorrhagiae	Birkini	Birkin	
18	*L. interrogans*	Icterohaemorrhagiae	Gem	Simon	
19	Unknown	Icterohaemorrhagiae	Hongchon	18 R	Kmety & Dikken,1993
20	*L. interrogans*	Icterohaemorrhagiae	Mankarso	Mankarso	Brenner *et al*. 1999
21	*L. interrogans*	Icterohaemorrhagiae	Naam	Naam	
22	*L. interrogans*	Icterohaemorrhagiae	Smithi	Smith	
23	*L. borgpetersenii*	Icterohaemorrhagiae	Tonkini	LT 96-68	
24	Unknown	Icterohaemorrhagiae	Yeonchon	HM 3	Kmety & Dikken,1993
25	Unknown	Sarmin	Cuica	RP 88	
26	*L. santarosai*	Sarmin	Machiguenga	MMD 3	Brenner *et al*.,1999
27	*L. santarosai*	Sarmin	Rio	Rr 5	
28	*L. weilii*	Sarmin	Sarmin	Sarmin	
29	*L. interrogans*	Sarmin	Waskurin	LT 63-68	
30	*L. santarosai*	Sarmin	Weaveri	CZ 390	

Table 2. Cross agglutination absorption test (CAAT) between selected reference serovars of the Icterohaemorrhagiae group and serovar Sokoine (RM1 isolate).

Antiserum against serovar:	Absorbing serovar/strain	Homologous titre before absorption	Homologous titre after absorption	Remaining homologous titre in percentage
Sokoine	Birkini	1:10240	1:5120	50 %
	Bogvere	1:10240	1:5120	50 %
	Gem	1:10240	1:5120	50 %
	Lai	1:10240	1:5120	50 %
	Ndahambukuje	1:10240	1:5120	50 %
	Ndambari	1:10240	1:160	1.5 % *
	Mwogolo	1:10240	1:5120	50 %
Birkini	Sokoine	1:2560	1:1280	50 %
Bogvere		1:5120	1:1280	24.3 %
Gem		1:2560	1:1280	50 %
Lai		1:5120	Not tested	Not tested
Ndahambukuje		1:2560	1:320	12.5 %
Ndambari		1:1280	1:640	50 %

Homologous titre of < 10 % remaining in both forward and reverse reactions means same serovars. Serovar Sokoine (1.5 %) and Ndambari (50 %) are therefore different as one has > 10 % homologous antibodies remaining.

Fig.1. Determination of the pathogenic status of isolate RM1 (serovar Sokoine) by PCR. PCR products (330 bp) of RM1, other isolates and *Leptospira* reference strains were generated using Lepat 1 and Lepat 2 primers for detection of pathogenic leptospires. M- DNA size marker (100-1000 bp), RM1 isolate (lane 1), RM4 isolate (lane 2), RM7 isolate (lane 3), Sh9 isolate (lane 4), Sh25 isolate (lane 5), serogroup Semaranga serovar Semaranga, strain Veldrat Semarang (lane 6), serogroup Icterohaemorrhagiae serovar Mwogolo, strain Mwogolo (lane 7). The PCR products were separated by electrophoresis in 3 % agarose and stained with ethidium bromide.

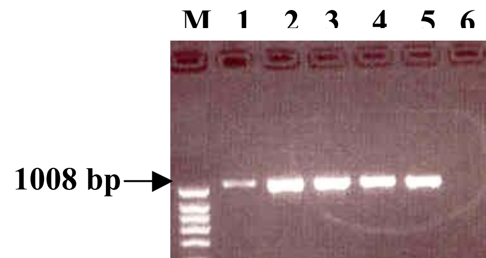

Fig.2. Establishment of the pathogenic status of isolate RM1 (serovar Sokoine) by PCR. PCR products (1008 bp) of RM1, other isolates and the reference strains were obtained with LP1 and *a*1190 primers for pathogenic leptospires. DNA size marker, 100-1000 bp (M), isolates RM1 (lane 1), RM4 (lane 2), RM7 (lane 3), Sh9 (lane 4), pathogenic *Leptospira* serovar Mwogolo, strain Mwogolo (lane 5) and saprophytic serovar Semaranga, strain Veldrat Semarang (lane 6). PCR products were separated by electrophoresis in 1.5 % agarose gel and stained with ethidium bromide.

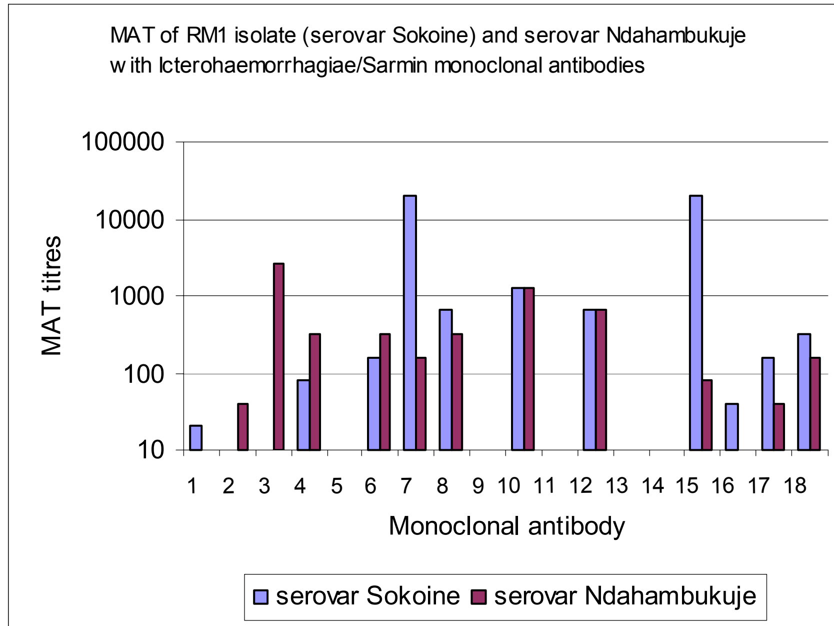

Fig.3. Serotyping of leptospires with monoclonal antibodies (mAbs). Comparison of the histograms of the RM1 isolates (serovar Sokoine) and reference serovar Ndahambukuje. The y-axis indicates reciprocal agglutination titres in the MAT with the 18 mAbs in a log scale; the x-axis indicates the following mAbs: 1-18 (F12C3-10, F20C3, F20C4-1, F52C1, F52C2, F70C4-1, F70C7-8, F70C13-1, F70C14-6, F70C20-3, F70C24-14, F70C26-1, F82C1-3, F82C2-2, F82C7-3, F82C8-4, F89C3-3, and F89C12-4).

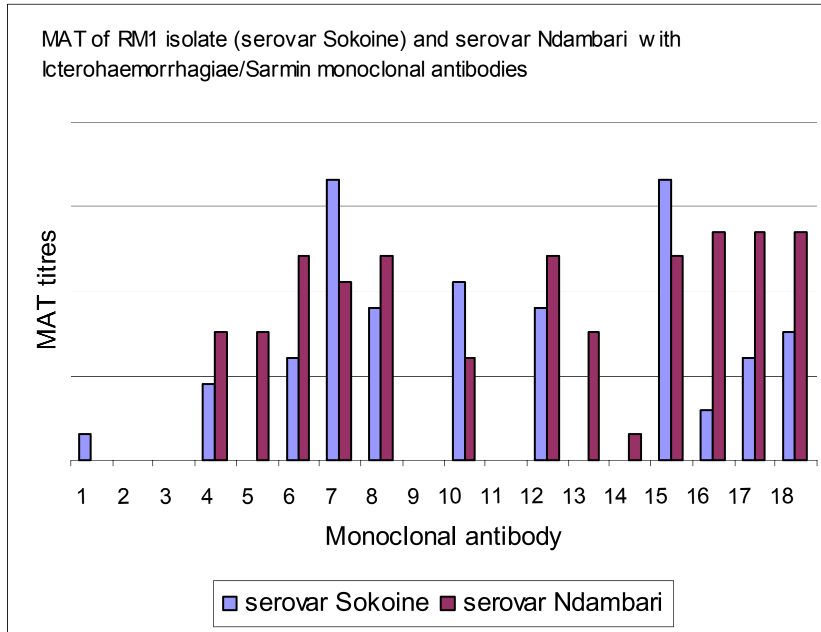

Fig.4. Serotyping of leptospires with monoclonal antibodies (mAbs). Comparison of the histograms of the RM1 isolates (serovar Sokoine) and reference serovar Ndambari. The y-axis indicates reciprocal agglutination titres in the MAT with the 18 mAbs in a log scale; the x-axis indicates the mAbs used as follows: 1-18 (F12C3-10, F20C3, F20C4-1, F52C1, F52C2, F70C4-1, F70C7-8, F70C13-1, F70C14-6, F70C20-3, F70C24-14, F70C26-1, F82C1-3, F82C2-2, F82C7-3, F82C8-4, F89C3-3, and F89C12-4).