Molecular characterization of *Leishmania* species from stray dogs and human patients in Saudi Arabia

Abdullah D. Alanazi 1 · Abdulazi S. Alouffi 2 · Mohamed S. Alyousif 3 · Abdulsadah A. Rahi 4 · Hend H. A. M. Abdullah 5 · Fabio A. Brayner 6 · Jairo Alfonso Mendoza-Roldan 7 · Marcos Antonio Bezerra-Santos 7 · Domenico Otranto 7,8

Received: 8 February 2021 / Accepted: 18 April 2021 / Published online: 4 May 2021 © The Author(s) 2021

**Abstract**

*Leishmania major* and *Leishmania tropica* cause cutaneous leishmaniasis in humans and dogs in several parts of the world, with a large number of cases recorded in the Middle East. However, when they occur in sympatry, the role of each species of *Leishmania* in the epidemiology of cutaneous leishmaniasis (CL) is not clear. To assess the frequency and to identify the species of *Leishmania* that infect humans and stray dogs in Riyadh and Al-Qaseem (Saudi Arabia), 311 stray dogs and 27 human patients who were suspected for *Leishmania* infection were examined for CL by a nested polymerase chain reaction (nPCR). Seven (25.9%) out of 27 human patients scored positive for *Leishmania* spp. (i.e., *L. major* in five patients from Riyadh and *L. tropica* in two patients from Al-Qaseem). Out of 311 dogs, five (1.6%) were infected by *L. tropica*. Data herein presented demonstrate the occurrence of *L. tropica* in dogs and humans in Saudi Arabia, as well as the occurrence of *L. major* in humans.

**Keywords** *Leishmania tropica* · *Leishmania major* · Dogs · Patients · kDNA · nPCR · Saudi Arabia

**Introduction**

Leishmaniases are a complex group of diseases caused by protozoa of the genus *Leishmania*, which are included in the group of neglected tropical diseases affecting mainly vulnerable human populations worldwide (WHO 2018). *Leishmania* spp. are transmitted by phlebotomine sand flies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Dantas-Torres et al. 2012). The disease caused by these protozoa is classified as cutaneous (CL), visceral (VL), and mucocutaneous (MCL) leishmaniases, all of which have been reported in Saudi Arabia (Abuzaid et al. 2017; Sirdar et al. 2018; Hawash et al. 2018). Moreover, CL caused by *Leishmania major* has been reported in that country, with the highest prevalence being recorded in the Riyadh, Qassim, Al-Madinah, Al-Hassa, Hail, and Asir regions (Al-Tawfiq and AbuKhamsin 2004; Amin et al. 2013; Alanazi et al. 2016), where more than 26,300 cases had been estimated from 2006 to 2016 (Abuzaid et al. 2017). In addition, there are several reports of leishmaniases caused by *Leishmania*...
infantum, L. major, and Leishmania tropica in humans and wild animals in Saudi Arabia (Peters et al. 1986; Elbihari et al. 1987; Al-Zahrani et al. 1988a, 1988b; Alanazi et al. 2019a, 2019b).

In western Saudi Arabia (Al-Madinah Al-Munawarah province), CL was diagnosed in human patients by internal transcribed spacer 1 (ITS-1), polymerase chain reaction (PCR), and restriction fragment length polymorphism (RFLP) (El-Beshbishy et al. 2013a, 2013b). The PCR screening established L. major and L. tropica as the causative agents for the above infection, with a kDNA PCR sensitivity of 90.7% and of ITS-1 PCR of 70.1%. Additionally, Leishmania spp. were detected in human patients in Al-Qaseem province, central Saudi Arabia, with prevalence of 49.5% for L. major, 28.6% for L. tropica, and 3.9% for L. infantum (Rasheed et al. 2019).

Despite the availability of several molecular studies that report the diagnosis and identification of Leishmania species worldwide (Ferreira et al. 2008; Akhavan et al. 2010; Toz et al. 2013; Silva et al. 2017), in Saudi Arabia, information on CL in human patients and on dog populations from endemic areas is still scant. Therefore, the aim of this study is to detect and identify the Leishmania spp. infecting humans and stray dogs in Al-Qaseem province and in Riyadh city, Saudi Arabia, providing data for a better understanding of the epidemiology of the infection in the study area.

Material and methods

Study areas, sampling, and DNA isolation

The investigation was conducted from January 2018 to May 2019 in Al-Qaseem province (latitude 25–23° N and longitude 42–24° E) and Riyadh city (latitude 24–08°N and longitude 47–18° E), Saudi Arabia (Fig. 1). A total of 27 human patients who were suspected to be infected by Leishmania species were seen in either the King Saud Medical City in Riyadh city (n =16) or Buraydah Central Hospital (n =11) in Al-Qaseem province. The presence of Leishmania was investigated in all samples, which were collected after clinical examination (Akilov et al. 2007). Briefly, skin biopsies (i.e., 5–10 mm in diameter) were taken under sterile conditions from the border of the ulcerous and cutaneous lesions, and DNA samples were extracted from all biopsies by MagNaA pure DNA extraction through the use of a Pure LC DNA Isolation Kit (Roche Applied Science, Germany) according to the manufacturer’s instructions.

From January 2018 to May 2019, 311 stray dogs were trapped in Al-Qaseem province by bait traps (Havahart®) and were examined physically for canine leishmaniasis skin lesions. Seven dogs were suspected of infection with canine leishmaniasis due to the presence of cutaneous nodules or ulcerated lesions. Skin biopsies (5mm in diameter) were collected under sterile conditions from the borders of the ulcers and were inoculated into M199 medium (Gibco, Life Technologies, Germany), which was supplemented with 25 mmol/l of 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (pH:7.5) and 20% fetal bovine serum (Gibco, Life Technologies, Germany). These samples were then incubated at 24 °C. Ten days after sample incubation, parasites were harvested and washed with ice-cold phosphate-buffered saline (10X PBS, pH: 7.4) and stored at −20°C before DNA isolation. DNA from parasite culture was isolated by use of the ReliaPrep™ gDNA Tissue Miniprep System Kit (Promega, Madison, USA), following the manufacturer’s instructions.

Leishmania-nested PCR

DNA samples from humans and dogs were screened via nested PCR. Initial amplification was performed with the
The amplified products of \textit{Leishmania} sequencing and BLAST analysis

primers (CSB2XF: 5’-ATTTTTCCGCGATTTTCGCAG AAACG-3’) and (CSB1XR: 5’-CGAGTCAGCAGAAC TCCCGTCTTCA-3’). The set of primers (13Z: 5’-ACTGGGTTGGTGAATAATG-3’) and (LiR: 5’-TCGG AGAACGCCCT-3’) were applied for the second step (Noyes et al. 1998). These primers amplify kDNA fragments of ~680bp for \textit{L. infantum}, ~750bp for \textit{L. tropica}, and ~560bp for \textit{L. major} (Noyes et al. 1998).

The preparation of the PCR master mix was performed using the AccuPower® PCR PreMix kit (Bioneer, Daejon, Korea). The prepared PCR pre-mix volumes containing potassium chloride (KCl) at a concentration of 30mM, magnesium chloride (MgCl2) at 1.5mM, tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCL at pH 9.0) at 10mM, Taq DNA polymerase, and deoxynucleoside triphosphate (dNTP) were adjusted to 2μl. In addition, 1μl of each initial CSB2XF and CSB1XR primers at concentrations of 10pmol/μl (Bioneer, Daejon, Korea) and 3μl of DNA were added to the reaction mixture. Finally, 13μl of deionized water (ddH2O) were added up to a total volume of 20μl for reaction. Negative control was included in the final nPCR. The reaction was performed in a thermal cycler (Techne TC-3000, USA) according to the following conditions: initial denaturation temperature of 94°C for 5min, 30 cycles of denaturation at 94°C for 30s, annealing at 55°C for 60s, extension at 72°C for 60s, and final extension at 72°C for 7min; and then the reaction was held at 4°C.

The second step of PCR involved 13Z and LiR primers, and the same PCR master mix except that 3μl of template PCR product from the first reaction was used. In this second round, PCR products that were obtained were electrophoresed on a 1.5% agarose gel containing 1μl Syber safe (Thermo Scientific™, Nalgene, UK) in tris-acetate–ethylenediaminetetraacetic acid (EDTA) buffer (50X) at 100V for 45min and visualized under a UV imaging system (ImageQuant Laz4000, GE Healthcare Life Science, Hammersmith, UK).

**Leishmania kDNA sequencing and BLAST analysis**

The amplified products of \textit{Leishmania} species were sequenced, and the results were compared with the sequences available in the GenBank database via BLAST search tool (http://blast.ncbi.nlm.nih.gov/). The obtained sequences were aligned with a set of reference sequences that were available in GenBank using CLUSTALW in MEGA software version 7.0 (Kumar et al. 2016). The phylogenetic tree was constructed using the maximum-likelihood method and with the Hasegawa-Kishino-Yano (HKY) model with 2000 bootstrap replicates in MEGA 7.0 software, using \textit{Trypanosoma cruzi} (AJ748063) as outgroup (Kumar et al. 2016; Al-Bajalan et al. 2018).

**Results**

Of the 27 human patients who were examined, five out of 16 (31.2%) from Riyadh and two out of 11 (18.2%) from Al-Qaseem were positive for \textit{Leishmania} spp. Sequence analysis of the \textit{Leishmania} kDNA confirmed that the five positive human samples from Riyadh were all \textit{L. major} with nucleotide identity ranging from 99.3 to 100% with \textit{L. major} sequences from Iraq (MN313423). The \textit{Leishmania} sequences from the two-positive human samples from Al-Qaseem presented identity of 99.7 to 100% with \textit{L. tropica} from Iraq (MF166799).

Of 311 dogs, seven (2.3%) presented cutaneous lesions (i.e., 1.5 × 5 cm) in different anatomical sites (e.g., nose, muzzle, abdomen, and interdigital spaces), and five of them were positive for \textit{L. tropica} (Table 1), presenting nucleotide identity ranging from 99.33 to 99.80% with sequences of \textit{L. tropica} from Iraq (MF166800, MN334661) and the UK (AF308689).

The phylogenetic tree for \textit{L. major} and \textit{L. tropica} sequences from human samples clustered with sequences from Iraq, and those for \textit{L. tropica} from dog samples clustered with sequences from Iraq and the UK (Fig. 2). In addition, the isolates of \textit{L. tropica} that were taken from humans and dogs in the present study were closely related (i.e., 98.60 to 99.65% with query cover that ranged from 98.20 to 99.50%) to \textit{kDNA} sequences from Iraq (MN313423). The \textit{Leishmania} sequences from human samples from Al-Qaseem presented identity of 99.7 to 100% with \textit{L. tropica} from Iraq (MF166799).

The phylogenetic tree for \textit{L. major} and \textit{L. tropica} sequences from human samples clustered with sequences from Iraq, and those for \textit{L. tropica} from dog samples clustered with sequences from Iraq and the UK (Fig. 2). In addition, the isolates of \textit{L. tropica} that were taken from humans and dogs in the present study were closely related (i.e., 98.60 to 99.65% with query cover that ranged from 98.20 to 99.50%) to \textit{kDNA} sequences of \textit{L. tropica} from Iraq (MF166800, MN334661) and the UK (AF308689).

The phylogenetic tree for \textit{L. major} and \textit{L. tropica} sequences from human samples clustered with sequences from Iraq, and those for \textit{L. tropica} from dog samples clustered with sequences from Iraq and the UK (Fig. 2). In addition, the isolates of \textit{L. tropica} that were taken from humans and dogs in the present study were closely related (i.e., 98.60 to 99.65% with query cover that ranged from 98.20 to 99.50%) to \textit{kDNA} sequences of \textit{L. tropica} from Iraq (MF166800, MN334661) and the UK (AF308689).

**Discussion**

This study provides molecular evidence of the circulation of \textit{L. major} and \textit{L. tropica} in human and dog populations from the investigated areas. The above \textit{Leishmania} spp. have already been recorded as agents of cutaneous leishmaniasis in Saudi Arabia and Middle Eastern countries (Bamorovat et al. 2015; Al-Salem et al. 2016; Baneth et al. 2016; Al-Bajalan et al. 2018). However, here we provide data on the occurrence of \textit{L. tropica} in humans and dogs from the central part of Saudi Arabia. This infection has been previously reported in the west and south west of Saudi Arabia in association with the distribution of \textit{Phlebotomus sergenti}, a proper vector for \textit{L. tropica} (Al-Zahrani et al. 1988a, 1988b). Conversely, \textit{L. major} is more prevalent throughout the country and can
be found in the open desert regions of Saudi Arabia (Abuzaid et al. 2017; Haouas et al. 2017). Previous studies performed in Saudi Arabia have reported the natural infection by *L. major* in dogs through the use of enzymatic biochemical methods (Elbihari et al. 1984; Peters et al. 1985), though in these studies, no clinical information was available nor were serology or molecular confirmation performed.

The high similarities of the nucleotides of human *L. major* and *L. tropica* isolates with those of Iraq (accession numbers MN313423 and MF166799) and of the dog *L. tropica* isolates

| Dog ID  | Site of trapped dogs | Sex | Age (years) | Location of cutaneous lesions | Leishmania tropica |
|---------|----------------------|-----|-------------|-------------------------------|-------------------|
| Dog No.1 | Unayzah              | Male| 2           | Left ear                      | Neg               |
| Dog No.2 | Al Bukayriyah        | Male| 1           | Nose                          | Pos               |
| Dog No.3 | Buraydah             | Male| 4           | Abdomen                       | Neg               |
| Dog No.4 | Al Bukayriyah        | Female| 2    | Under mouth lips              | Pos               |
| Dog No.5 | Buraydah             | Male| 3           | Muzzle                        | Pos               |
| Dog No.6 | Ar Rass              | Female| 3    | Nose                          | Pos               |
| Dog No.7 | Buraydah             | Male| 2           | Upper right leg               | Pos               |

Fig. 2 Maximum likelihood Kinetoplast DNA-based phylogenetic analysis of genotypes identified in this study. Phylogenetic tree highlights the position of *Leishmania* spp. of the present study (bold) using 2000 bootstrap replicates and *Trypanosoma cruzi* as outgroup
with those of Iraq and UK (accession numbers MN334665, MF166799, and AF308689, respectively) were confirmed by phylogenetic analysis. Moreover, this study showed that *L. tropica* from humans and dogs was closely related with the kDNA of *L. tropica* samples from the Middle East. This might be due to the distribution of similar sand fly species in the different parts of Saudi Arabia and the Middle East, which may act as proper vectors of both *Leishmania* spp. (Al-Salem et al. 2016; Du et al. 2016). In the phylogenetic tree, *L. tropica* and *L. major* clustered in separate clades, distinct from the *L. donovani* complex (i.e., *L. infantum* and *L. donovani*). Moreover, *L. tropica* sequences presented very limited intra-specific genetic diversity, unlike the sequences that were previously classified as belonging to the *L. donovani* complex (Medkour et al. 2020).

Though CL is endemic in many parts of Saudi Arabia, the paucity of data concerning the relationship between the disease, the vectors, and the reservoirs is a major hindrance to the comprehension of the transmission cycles, particularly given that the distribution patterns can easily change through the years in specific geographical areas (Mendoza-Roldan et al. 2020). Data herein reported contribute to the filling of existing gaps in knowledge to increase the awareness of the Ministry of Health in Saudi Arabia to prevent outbreaks and the spread of CL.

**Conclusion**

This study confirms the occurrence of *L. major* and *L. tropica* in humans and *L. tropica* in dogs from Al-Qaseem province and Riyadh City, Saudi Arabia. However, the relationship between sand fly vectors and reservoirs of this disease remains unclear, advocating further studies in these areas.

**Author contribution**

ADA, ASA, and AAR conceived the study. MSA and ASA performed field work. ASA collected patient and dog samples. ASA and ADA carried out the biopsy tissue collections and DNA extractions. MAA, AAR, and FAB performed laboratory work and analyzed data. HHAMA and ADA and JM-R performed phylogenetic analysis. ADA, MSA, JAMR, MABS, DO, and FAB wrote the first draft of the manuscript. JAMR, MABS, DO, and ADA reviewed and wrote the final draft of the manuscript. All authors read and approved the final version of the manuscript.

**Funding**

Open access funding provided by Università degli Studi di Bari Aldo Moro within the CRUI-CARE Agreement. This work was kindly supported by funding from a Researcher Supporting Project (RSP-2020-192), King Saud University, Riyadh, Saudi Arabia.

**Declarations**

This study was reviewed and approved by the Ethics Committee of the Department of Biological Sciences at the Shaqra University, according to the ethical principles of animal research (protocol SH 2-2017).

**Competing interests**

The authors declare no competing interests.

**Open Access**

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

Abuzaid AA, Abdooon AM, Aldahan MA, Alzahrani AG, Alhakeem RF, Asiri AM, Alzahrani MH, Memish ZA (2017) Cutaneous Leishmaniasis in Saudi Arabia: a comprehensive overview. Vector Borne Zoonotic Dis 17:673–684

Akhvan AA, Mirhendi H, Khamesipour A, Alimohammadian MH, Rassi Y, Bates P, Kamhawi S, Valenzuela JG, Arandian MH, Abdoli H (2010) *Leishmania* species: detection and identification by nested PCR assay from skin samples of rodent reservoirs. Exp Parasitol 126:552–556

Akirov OE, Khachemoune A, Hasan T (2007) Clinical manifestations and classification of Old World cutaneous leishmaniasis. Int J Dermatol 46:132–142

Alanzazi AD, Alyousif MS, Saifi M, Alanazi IO (2016) Epidemiological studies on cutaneous leishmaniasis in Ad-Dawadimi District, Saudi Arabia. Trop J Pharm Res 15:2709–2712

Alanzazi AD, Puschendorf R, Alyousif MS, Al-Khalifa MS, Alkharbi S, Al-Shehri Z, Alanazi I, Al-Mohammed HL, Ylaey Y (2019a) Molecular detection of *Leishmania* spp. in skin and blood of stray dogs from endemic areas of cutaneous Leishmaniasis in Saudi Arabia. Iran J Parasitol 14:231–239.

Alanzazi AD, Rahi AA, Ali AM, Alyousif MS, Alanazi IO, Mahmoud MS, Abdel-Shafy S, Alraey Y, Alouffi A (2019b) Molecular detection and phylogenetic analysis of *Leishmania major* in stray dogs in Riyadh Province, Saudi Arabia. Trop Biomed 36:315–323

Al-Bajalan MMM, Niranji SS, Al-Jaf SMA, Kato H (2018) First identification of *L. major* in a dog in an endemic area of human cutaneous leishmaniasis in Iraq: molecular and phylogenetic studies. Parasitol Res 117:585–590

Al-Salem WS, Pigott DM, Subramaniam K, Haines LR, Kelly-Hope L, Molyneux DH, Hay SI, Acosta-Serrano A (2016) Cutaneous Leishmaniasis and conflict in Syria. Emerg Infect Dis 22:931–933

Al-Tawfiq JA, AbuKhamis A (2004) Cutaneous leishmaniasis: a 46-year study of the epidemiology and clinical features in Saudi Arabia (1956–2002). Int J Infect Dis 8:244–250

Al-Zahrani M, Peters W, Evans D (1988a) Visceral leishmaniasis in man and dogs in south-west Saudi Arabia. Trans R Soc Trop Med Hyg 82:857

Al-Zahrani M, Peters W, Evans D, Chin C, Smith V, Lane R (1988b) *Phlebotomus sergenti*, a vector of *Leishmania tropica* in Saudi Arabia. Trans R Soc Trop Med Hyg 82:416

Amin TT, Al-Mohammed HI, Kaliyadan F, Mohammed BS (2013) Cutaneous leishmaniasis in Al Hassa, Saudi Arabia: epidemiological trends from 2000 to 2010. Asian Pac J Trop Biomed 6:667–672

Bamorovat M, Sharifi I, Dabiri S, Mohammadi MA, Harandi MF, Mohebali M, Afatoonian MR, Keyhani A (2015) *Leishmania*
tropica in stray dogs in southeast Iran. Iran J Public Health 44:1359–1366
Banhet G, Nachum-Biala Y, Simon MS, Brenner O, Gaier S, Rojas A, Yasar-Landau D (2016) Leishmania major infection in a dog with cutaneous manifestations. Parasit Vectors 9:246
Dantas-Torres F, Solano-Gallego L, Baneth G, Ribeiro VM, de Paiva-Cavalcanti M, Otranto D (2012) Canine leishmaniasis in the Old and New Worlds: unveiled similarities and differences. Trends Parasitol 28:531–538
Du R, Hotez PJ, Al-Salem WS, Acosta-Serrano A (2016) Old world cutaneous leishmaniasis and refugee crises in the Middle East and North Africa. PLoS Negl Trop Dis 10:e0004545
El-Beshbishy HA, Al-Ali KH, El-Badry AA (2013a) Molecular characterization of cutaneous leishmaniasis in Al-Madinah Al-Munawarah province, western Saudi Arabia. Int J Infect Dis 17:234–238
El-Beshbishy HA, Al-Ali KH, El-Badry AA (2013b) Molecular characterization of Leishmania infection in sand flies from Al-madinah Al-munawarah province, western Saudi Arabia. Exp Parasitol 134:211–215
Elbihari S, Cheema A, El-Hassan A (1987) Leishmaninia infecting man and wild animals in Saudi Arabia. 4. Canine cutaneous leishmaniasis in the Eastern Province. Trans R Soc Trop Med Hyg 81:925–927
Elbihari S, Cheema A, El-Hassan A (1987) Leishmania infecting man and wild animals in Saudi Arabia. 4. Canine cutaneous leishmaniasis in the Eastern Province. Trans R Soc Trop Med Hyg 81:925–927
Elbihari S, Kawasmeh Z, Al Naiem A (n.d.) Possible reservoir host (s) of Leishmania infections in stray dogs in southeast Iran. Iran J Public Health 44:1359–1366
Ferreira SA, Ituassu LT, Melo MN, Andrade AS (2008) Evaluation of the conjunctival swab for canine visceral leishmaniasis diagnosis by PCR-hybridization in Minas Gerais State, Brazil. Vet Parasitol 152:257–263
Haoous N, Amer O, Alishamrri FF, Al-Shammari S, Remadi L, Ashankyty J (2017) Cutaneous leishmaniasis in northwestern Saudi Arabia: identification of sand fly fauna and parasites. Parasit Vectors 10:544
Hawash YA, Ismail KA, Abdel-Wahab MM, Khalifa M (2018) Diagnosis, treatment and clinical features of cutaneous Leishmaniasis in Saudi Arabia. Korean J Parasitol 56:229–236
Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
Medkour H, Laidoudi Y, Athias E, Bouam A, Dizoé S, Davoust B, Medianikov O (2020) Molecular and serological detection of animal and human vector-borne pathogens in the blood of dogs from Côte d’Ivoire. Comp Immunol Microbiol Infect Dis 69:101412
Mendoza-Roldan J, Benelli G, Panarese R, Iatta R, Furlanello T, Beugnet F, Zatelli A, Otranto D (2020) Leishmania infantum and Dirofilaria immitis infections in Italy, 2009–2019: changing distribution patterns. Parasit Vectors 13:193
Noyes HA, Reburn H, Bailey JW, Smith D (1998) A nested-PCR-based schizodeme method for identifying Leishmania kinetoplast minicircle classes directly from clinical samples and its application to the study of the epidemiology of Leishmania tropica in Pakistan. J Clin Microbiol 36:2877–2881
Peters W, Elbihari S, Liu C, Le Blancq S, Evans D, Killick-Kendrick R, Smith V, Baldwin C (1985) Leishmania infecting man and wild animals in Saudi Arabia 1. General survey. Trans R Soc Trop Med Hyg 79:831–839
Peters W, Elbihari S, Evans D (1986) Leishmania arabica infecting man and wild animals in Saudi Arabia. 2. Leishmania arabica n. sp. Trans R Soc Trop Med Hyg 80:497–502
Rasheed Z, Ahmed AA, Salem T, Al-Dhubaibi MS, Al Robaee AA, Alzolibani AA (2019) Prevalence of Leishmania species among patients with cutaneous leishmaniasis in Qassim province of Saudi Arabia. BMC Public Health 19:384
Silva RC, Richini-Pereira VB, Kikuti M, Marson PM, Langoni H (2017) Detection of Leishmania (L.) infantum in stray dogs by molecular techniques with sensitive species-specific primers. Vet Q 37:23–30
Sirdar MK, Al-Zahrani MH, Dahlan AA, Sahli AA, Mohamed WS, Hejri YM, Dafalla OM, Alattass MS, Hayder A, Noureldin EM (2018) Epidemiology and incidence of leishmaniasis in Jazan region, Saudi Arabia (2007-2015): an overview. J Entomol Zool Stud 6:859–864
Toz SO, Culha G, Zeyrek FY, Ertabaklar H, Alkan MZ, Vardarl AR, Gunduz C, Ozbel Y (2013) A real-time ITS1-PCR based method in the diagnosis and species identification of Leishmania parasite from human and dog clinical samples in Turkey. PLoS Negl Trop Dis 7:e2205
WHO (2018) Report on the Interregional meeting on leishmaniasis among neighbouring endemic countries in the Eastern Mediterranean, African and Europ, Amman, 23–25 https://apps.who.int/iris/bitstream/handle/10665/311922/IC_Meet_Rep_2019_EN_20619.pdf?ua=1
Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.