Infection, Genetics and Evolution 76 (2019) 104073

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

ISC1, a new *Leishmania donovani* population emerging in the Indian sub-continent: Vector competence of *Phlebotomus argentipes*

Veronika Seblova[a], Jean-Claude Dujardin[b,c], Suman Rijal[d], Malgorzata Anna Domagalska[b,*], Petr Volf[a,*]

[a] Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
[b] Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[c] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
[d] BP Koirala Institute of Health Sciences, Dharan, Nepal

ARTICLE INFO

Keywords:
Leishmania donovani
Sand fly
Experimental development

ABSTRACT

Visceral leishmaniasis (VL), the most severe form of the disease, is caused by *Leishmania donovani* in the Indian sub-continent (ISC). Whole genome sequencing studies revealed that two parasite populations exist in the ISC: a main population named the Core Group (CG) found mostly in the lowlands, and a new, genetically different subpopulation called ISC1. Parasites belonging to the CG were shown to be responsible for the recent epidemics, while the ISC1 variant was originally identified in hilly districts of Nepal and was later on increasingly found in the lowlands. Importantly, the ISC1 and CG isolates differ in their drug susceptibility and virulence signatures, suggesting that ISC1 constitutes an emerging and functionally different variant of *L. donovani*. In present study we aimed to address the potential of ISC1 transmission by the natural vector of *L. donovani* in the lowlands, *Phlebotomus argentipes*. By experimental infection of sand flies with parasites of the different genotypes, we demonstrate that ISC1 and CG strains are developing similarly in *P. argentipes*, suggesting that *P. argentipes* is a fully competent vector for ISC1 parasites. Integration of previous and current findings shows thus that ISC1 is a new and different variant of *L. donovani*, fully adapted to spread in the ISC through the main vector. This information is directly useful for managers of the elimination program. Furthermore, integration of our successive studies (genotyping, phenotyping and vector competence) demonstrates the relevance of molecular surveillance and should be of interest for scientists working on vector borne diseases and control managers.

1. Introduction

Visceral leishmaniasis (VL) is due to 2 parasite species of the *Leishmania donovani* complex: *Leishmania infantum* around the Mediterranean basin and in Latin America and *Leishmania donovani* in the Indian subcontinent (ISC) and East Africa. It is the most severe form of the disease, being lethal in the absence of treatment. In 2012, global incidence was reported to range between 200,000 and 400,000 new VL cases each year, most of them in the ISC, followed by East-Africa (Alvar et al., 2012). In 2017, global incidence ranged between 50,000 and 90,000 (WHO, 2018), essentially because of a significant decrease of the cases in the ISC, while cases in East Africa were sustained (Burza et al., 2018). The reduction of cases in the ISC is attributed to the regional elimination programme that started in 2005 and aimed to reduce the disease burden to < 1 case per 10,000 population by 2020. However, it could also reflect the natural fluctuation of the disease, with epidemics reported every 15 years (Dye and Wolpert, 1988). Whatever the exact cause, new outbreaks will come and surveillance is more than ever required in the current 'post-elimination' phase (Rijal et al., 2019), including molecular surveillance through highly discriminatory tracking of the parasites to follow the evolution of epidemics, detect new variants and assess in real time the source of outbreaks (Domagalska et al., 2019).

We recently established the bases for such molecular surveillance by sequencing the whole genome of 200 *L. donovani* isolates from the region (Imamura et al., 2016): we discovered a main population in the lowlands (called the Core Group or CG) that drove the most recent epidemics in the ISC, but also a new subpopulation genetically quite different, called ISC1. This population clustered phylogenetically as an intermediate between ISC and East African *L. donovani*. It is currently unknown if ISC1 parasites are descendants of an old ISC population or if they were recently introduced from outside ISC. This new variant

* Corresponding authors.
E-mail addresses: mdomagalska@itg.be (M.A. Domagalska), volf@cesnet.cz (P. Volf).

https://doi.org/10.1016/j.meegid.2019.104073
Received 13 July 2019; Received in revised form 11 October 2019; Accepted 13 October 2019
Available online 17 October 2019

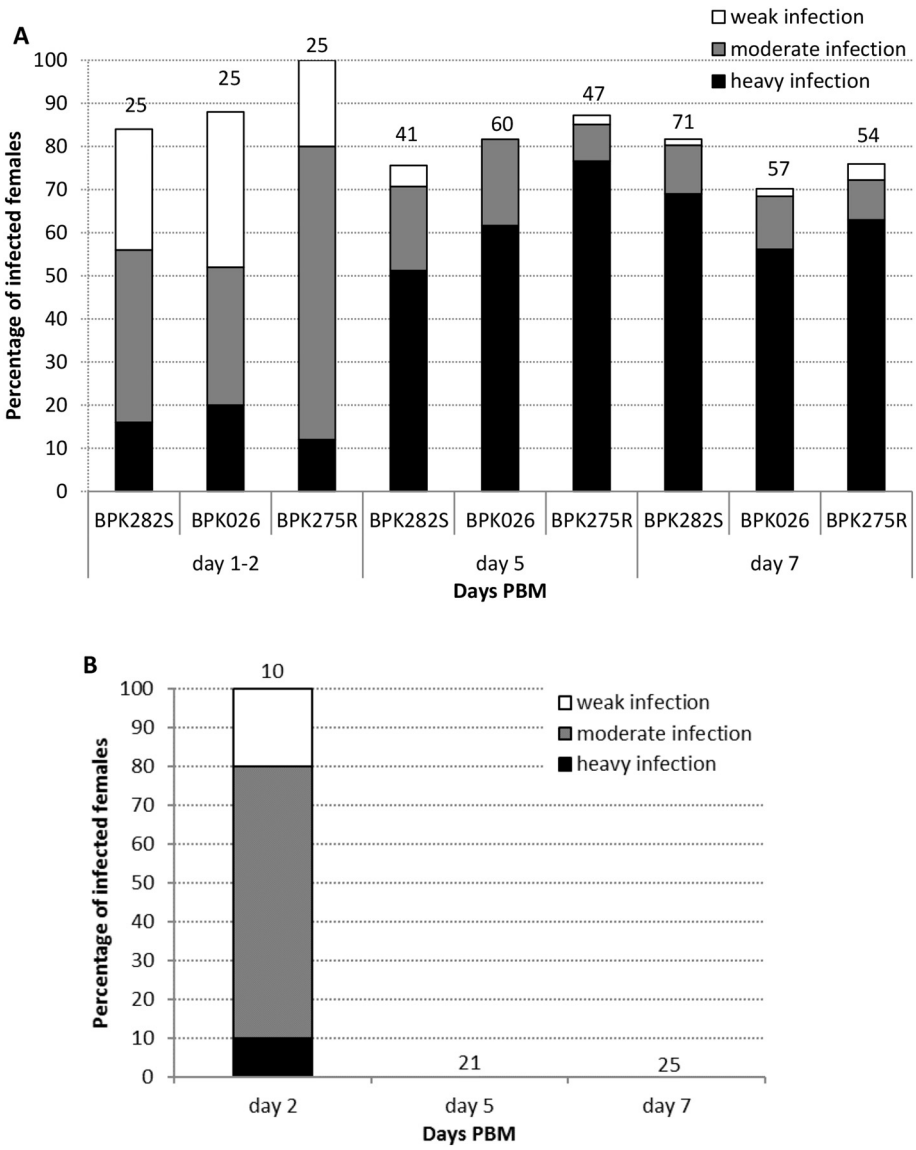

Fig. 1. A. Development of three *L. donovani* strains (strains BPK 282-S andc BPK 275-R, from CG and BPK026 from ISC1) in *P. argentipes*. Graph represents a summary of three experiments, there was no significant variation among replicates. Numbers above bars represent the number of sand fly females dissected. B. Development of *L. donovani* BPK 026 in *Phlebotomus papatasi*. Graph represents a summary of two experiments, there was no significant variation among replicates. Numbers above bars represent the number of sand fly females dissected.

originally encountered in patients originating from hilly districts of Nepal (Imamura et al., 2016), was later on increasingly found in the lowlands (Rai et al., 2017). In recent studies, we reported different signatures of drug susceptibility and virulence between ISC1 and CG parasites (Dumetz et al., 2018; Cuypers et al., 2018). ISC1 thus appears as an emerging and functionally different population of *L. donovani*. A particular attention should be given on its potential for further spreading. In this context, we aimed to study the potential of ISC1 transmission by the natural vector of *L. donovani* in the lowlands, *Phlebotomus argentipes,* and compare it with *P. papatasi*, the common peridomestic sand fly species in the Indian subcontinent and aggressive man-biter (reviewed by Dvorak et al., 2018) which was demonstrated as refractory to *L. donovani* (reviewed by Dostalova and Volf, 2012). This was done here by investigating in details the experimental development of CG and ISC1 strains of *Leishmania donovani* in the two sand fly species.

2. Materials and methods

Three clinical isolates of *L. donovani* were here used: (i) 2 CG ones representing the phenotypic diversity of that population (MHOM/NP/03/BPK282 and MHOM/NP/03/BPK275, respectively sensitive and resistant to antimonials (Rijal et al., 2007)) and (ii) MHOM/NP/02/BPK026 representing ISC1. The 3 lines were cryopreserved at the Institute of Tropical Medicine at low passage number (26, 37 and 20, respectively), thawn, briefly sub-cultivated on HOMEM and sent to Charles University. Two well-established laboratory colonies of sand flies were used: *P. argentipes,* originating from lowland India (Volf and Volfova, 2011), previously shown as highly susceptible to East-African *L. donovani* (Sadlova et al., 2017), and *P. papatasi*, originating from Cukurova plain near Adana, Turkey (Votypka et al., 2012), repeatedly shown as highly susceptible to various strains of *L. major* (Sadlova et al., 2010; Chajbullinova et al., 2012; Doehl et al., 2017). In each experiment, approximately 150 *P. argentipes* females (3-5 days old) were fed through a chick-skin membrane on heat-inactivated rabbit blood containing 10^6 promastigotes/ml. Engorged females were separated,

maintained at 26 °C, maintained at 26 °C under conditions described previously (Volf and Volfova, 2011) and dissected at days 1-2, 5, 7 post blood meal (PBM). Localization and intensity of *Leishmania* infection in sand fly midgut were estimated *in situ* under a light microscope, by scoring parasite loads as described by (Myskova et al., 2008): weak (< 100 parasites/gut), moderate (100-1000 parasites/gut) and heavy (> 1000 parasites/gut) infections. The experiment was repeated three times. Additionally, *P. papatasi* females (about 5 days old) were infected with ISC1 strain MHOM/NP/02/BPK026. Gut smears of *L. donovani*-infected females 7 days PBM were fixed with methanol, stained with Giemsa and examinated under the light microscope with an oil-immersion objective. Females of both species were at the same stage in the gonotrophic cycle 1-2, 5 and 7 days PBM, 1-2 referring to females with a bloodmeal surrounded by peritrophic membrane, while days 5-7 refer to females that already defecated a bloodmeal. Two hundred randomly selected promastigotes for each strain (from 1 to 2 sand flies/smears) were measured using ImageJ program. Body length, flagellar length and body width of parasites were measured for determination of metacyclogenesis of each strain (Rogers et al., 2002).

3. Results and discussion

On days 1-2 PBM, no differences were observed between the three *L. donovani* lines tested in *P. argentipes*. High infection rates (85–100%) were observed and most sand fly females harboured heavy or moderate parasite loads (Fig. 1A). Promastigotes were localized in the bloodmeal enclosed within the peritrophic matrix in the abdominal midgut. In late stage infections, on days 5 and 7 PBM, infection rates remain high (above 70% in all three strains) and no significant differences in infection intensities were observed between Sb-resistant and Sb-susceptible strains (Fig. 1A). Promastigotes migrated anteriorly to the thoracic midgut and colonized the stomodeal valve in majority of infected females (58% for BPK282, 80% for BPK026 and 56% for BPK275). All studied strains of *L. donovani*, both CG and ISC1, thrive in *P. argentipes*. A high number of parasites colonizing the stomodeal valve and the thoracic part of sand fly midgut are the important prerequisites for successful transmission (reviewed by Bates, 2008). All tested strains produced mature late-stage infections with presence of 10,5%, 10% and 15% metacyclics for BPK282, BPK026 and BPK275 strains, respectively. This demonstrated that ISC1 and CG strains are developing similarly in *P. argentipes* and suggested that *P. argentipes* is a fully competent vector also for ISC1 strains.

In the ISC, it is common to find *P. papatasi* in sympatry with *P. argentipes* (Burniston et al., 2010). The former species is the specific vector of *Leishmania major* and is refractory to *L. donovani* (reviewed by Kamhawi, 2006). This was verified here by infecting female *P. papatasi* with the ISC1 strain. On day 2, 100% of dissected females were infected and parasites were localized in the bloodmeal surrounded by peritrophic matrix. However, all 46 females dissected on days 5 and 7 were parasite-negative. (Fig. 1B). This demonstrated that the ISC1 strain is not able to develop late-stage infections in *P. papatasi* and confirmed that this widely-spread peridomestic and antropophilic sand fly species is not involved in circulation of ISC1 strains.

P. argentipes is well-known as the VL vector in the lowlands of ISC and insects morphologically identified as *P. argentipes* were reported to be infected with *L. donovani* in hilly districts of Nepal (Ostyn et al., 2015). Importantly, *P. argentipes* is known to be a permissive vector susceptible to various *Leishmania* species (reviewed by Dostalova and Volf, 2012). As such, it could play a major role in the colonization of ISC by new species or genetic variants. In present study, we demonstrated experimentally that the emerging ISC1 variant of *L. donovani* could develop as well as the CG strains that were responsible for the previous epidemics of VL in the region. This work should be complemented with finding of naturally infected *P. argentipes* during the field research and calls for more intensive control of this important vector.

Funding

This work was funded by the European Commission (Kaladrug project, grant EC-FP-222895) and ERD Funds, project CePaViP (16_019/0000759).

References

Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., Boer, M.D., The, W.H.O.L.C.T, 2012. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 7, e35671. https://doi.org/10.1371/journal.pone.0035671.

Bates, P.A., 2008. *Leishmania* sand fly interaction: progress and challenges. Curr. Opin. Microbiol. 11, 340–344. https://doi.org/10.1016/j.mib.2008.06.003.

Burniston, I., Roy, L., Picado, A., Das, M., Rijal, S., Rogers, M., Coosemans, M., Boelaert, M., Davies, C., Cameron, M., 2010. Development of an enzyme-linked immunosorbent assay to identify host-feeding preferences of *Phlebotomus* species (Diptera: Psychodidae) in endemic foci of visceral leishmaniasis in Nepal. J. Med. Entomol. 47, 902–906. https://doi.org/10.1603/me09184.

Burza, S., Croft, S.L., Boelaert, M., 2018. Leishmaniasis. Lancet 392, 951–970. https://doi.org/10.1016/S0140-6736(18)31204-2.

Chajbullinova, A., Votypka, J., Sadlova, J., Kvapilova, K., Seblova, V., Kreisinger, J., Jirku, M., Sanjoba, C., Gantuya, S., Matsumoto, Y., Volf, P., 2012. The development of *Leishmania turanica* in sand flies and competition with *L. major*. Parasit. Vectors 5, 219. https://doi.org/10.1186/1756-3305-5-219.

Cuypers, B., Berg, M., Imamura, H., Dumetz, F., De Muylder, G., Domagalska, M.A., Rijal, S., Bhattarai, N.R., Maes, I., Sanders, M., Cotton, J.A., Meysman, P., Laukens, K., Dujardin, J.-C., 2018. Integrated genomic and metabolomic profiling of ISC1, an emerging *Leishmania donovani* population in the Indian subcontinent. Infect. Genet. Evol. 62, 170–178. https://doi.org/10.1016/j.meegid.2018.04.021.

Doehl, J.S.P., Sadlova, J., Aslan, H., Pruzinova, K., Metangmo, S., Votypka, J., Kamhawi, S., Volf, P., Smith, D.F., 2017. *Leishmania* HASP and SHERP genes are required for in vivo differentiation, parasite transmission and virulence attenuation in the host. PLoS Pathog. 13, e1006130. https://doi.org/10.1371/journal.ppat.1006130.

Domagalska, M.A., Volf, P., Dujardin, J.-C., 2019. Post-elimination surveillance of visceral leishmaniasis in South-Asia: the need for molecular surveillance. B.M.J rapid response. https://www.bmj.com/content/364/bmj.k5224/rr.

Dostalova, A., Volf, P., 2012. *Leishmania* development in sand flies: parasite-vector interactions overview. Parasit. Vectors 5, 276. https://doi.org/10.1186/1756-3305-5-276.

Dumetz, F., Cuypers, B., Imamura, H., Zander, D., D'Haenens, E., Maes, I., Domagalska, M.A., Clos, J., Dujardin, J.-C., De Muylder, G., 2018. Molecular Preadaptation to Antimony Resistance in *Leishmania donovani* on the Indian Subcontinent. mSphere 3https://doi.org/10.1128/mSphere.00548-17. (pii: e00548-17).

Dvorak, V., Shaw, J., Volf, P., 2018. Parasite Biology: The Vectors. In: Bruschi, F., Gradoni, L. (Eds.), The Leishmaniases Old Neglected Tropical Diseases. Springer International Publishing, AG, pp. 31–77. https://doi.org/10.1007/978-3-319-72386-0. ISBN 978-3-319-72385-3.

Dye, C., Wolpert, D.M., 1988. Earthquakes, influenza and cycles of Indian kala-azar. Trans. R. Soc. Trop. Med. Hyg. 82, 843–850.

Imamura, H., Downing, T., Van den Broeck, F., Sanders, M.J., Rijal, S., Sundar, S., Mannaert, A., Vanaerschot, M., Berg, M., De Muylder, G., Dumetz, F., Cuypers, B., Maes, I., Domagalska, M., Decuypere, S., Rai, K., Uranw, S., Bhattarai, N.R., Khanal, B., Prajapati, V.K., Sharma, S., Stark, O., Schönian, G., De Koning, H.P., Settimo, L., Vanhollebeke, B., Roy, S., Ostyn, B., Boelaert, M., Maes, L., Berriman, M., Dujardin, J.C., Cotton, J.A., 2016. Evolutionary genomics of epidemic visceral leishmaniasis in the Indian subcontinent. elife 5https://doi.org/10.7554/eLife.12613.001. (pii: e12613).

Kamhawi, S., 2006. Phlebotomine sand flies and *Leishmania* parasites: friends or foes? Trends Parasitol. 22, 439–445. https://doi.org/10.1016/j.pt.2006.06.012.

Myskova, J., Votypka, J., Volf, P., 2008. *Leishmania* in sand flies: comparison of quantitative polymerase chain reaction with other techniques to determine the intensity of infection. J. Med. Entomol. 45, 133–138. https://doi.org/10.1603/0022-2585(2008)45[133:lisfco]2.0.co;2.

Ostyn, B., Uranw, S., Bhattarai, N.R., Das, M.L., Rai, K., Tersago, K., Pokhrel, Y., Durnez, L., Marasini, B., Van der Auwera, G., Dujardin, J.C., Coosemans, M., Argaw, D., Boelaert, M., Rijal, S., 2015. Transmission of *Leishmania donovani* in the hills of Eastern Nepal, an outbreak investigation in Okhaldhunga and Bhojpur Districts. PLoS Negl. Trop. Dis. 9, e0003966. https://doi.org/10.1371/journal.pntd.0003966.

Rai, K., Bhattarai, N.R., Vanaerschot, M., Imamura, H., Gebru, G., Khanal, B., Rijal, S., Boelaert, M., Pal, C., Karki, P., et al., 2017. Single locus genotyping to track *Leishmania donovani* in the Indian subcontinent: application in Nepal. PLoS Negl. Trop. Dis. 11, e0005420. https://doi.org/10.1371/journal.pntd.0005420.

Rijal, S., Yardley, V., Chappuis, F., Decuypere, S., Khanal, B., Singh, R., Boelaert, M., De Doncker, S., Croft, S., Dujardin, J.C., 2007. Antimonial treatment of visceral leishmaniasis: are current in vitro susceptibility assays adequate for prognosis of in vivo therapy outcome? Microbes Infect. 9, 529–535. https://doi.org/10.1016/j.micinf.2007.01.009.

Rijal, S., Sundar, S., Mondal, D., Das, P., Alvar, J., Boelaert, M., 2019. Eliminating visceral leishmaniasis in South Asia: the road ahead. B.M.J 364https://doi.org/10.1136/bmj.k5224. k5224.

Rogers, M.E., Chance, M.L., Bates, P.A., 2002. The role of promastigote secretory gel in the origin and transmission of the infective stage of *Leishmania mexicana* by the sandfly *Lutzomyia longipalpis*. Parasitology 124, 495–507 (no doi available).

Sadlova, J., Price, H.P., Smith, B.A., Votypka, J., Volf, P., Smith, D.F., 2010. The stage-regulated HASPB and SHERP proteins are essential for differentiation of the protozoan parasite *Leishmania major* in its sand fly vector, *Phlebotomus papatasi*. Cell. Microbiol. 12, 1765–1779. https://doi.org/10.1111/j.1462-5822.2010.01507.x.

Sadlova, J., Myskova, J., Lestinova, T., Votypka, J., Yeo, M., Volf, P., 2017. *Leishmania donovani* development in *Phlebotomus argentipes*: comparison of promastigote- and amastigote-initiated infections. Parasitology 144, 403–410. https://doi.org/10.1017/S0031182016002067.

Volf, P., Volfova, V., 2011. Establishment and maintenance of sand fly colonies. J. Vector Ecol. 36, S1–S9. https://doi.org/10.1111/j.1948-7134.2011.00106.x|.

Votypka, J., Kasap, O.E., Volf, P., Kodym, P., Alten, B., 2012. Risk factors for cutaneous leishmaniasis in Cukurova region, Turkey. Trans. R. Soc. Trop. Med. Hyg. 106, 186–190. https://doi.org/10.1016/j.trstmh.2011.12.004.

WHO, 2018. Leishmaniasis. World Health Organization, Geneva. http://www.who.int/mediacentre/factsheets/fs375/en/ (accessed April 15, 2019).