Short Communication

The Development of *Leishmania tropica* in Sand Flies (Diptera: Psychodidae): A Comparison of Colonies Differing in Geographical Origin and a Gregarine Coinfection

MAGDALENA JANCAROVA,[1] JANA HLAVACOVA, and PETR VOLF

Department of Parasitology, Faculty of Science, Charles University, Vinicna 7, Prague 2, 128 44, Czech Republic.

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ABSTRACT *Phlebotomus sergenti* Parrot, 1917 is the main vector of *Leishmania tropica*; however, its broad geographical range and molecular heterogeneity suggest possible variability in vector competence. We infected laboratory-reared *P. sergenti* originating from Turkey and Israel to compare their susceptibility to *L. tropica*. In both tested groups, heavy late-stage infections with the presence of metacyclic forms and colonization of the stomodeal valve were observed. The similar development of *Leishmania* in both sand fly colonies indicates that the different geographical origin of *P. sergenti* is not reflected by a different vector competence to *L. tropica*. Additionally, we tested the effect of the gregarine *Psychodiella sergenti* on *L. tropica* coinfections; no apparent differences were found between *P. sergenti* infected or not infected by gregarines.

KEY WORDS *Phlebotomus sergenti*, *Leishmania tropica*, vector competence, coinfection, gregarine

Leishmaniases are vector-borne diseases with a wide range of clinical outcomes. Their causative agents are parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) transmitted by the bite of phlebotomine sand flies (Diptera: Psychodidae). *Leishmania tropica* causes cutaneous leishmaniasis in many countries around the Mediterranean basin, the Middle East, Central Asia, and East Africa. The primary specific vector is *Phlebotomus sergenti* Parrot, 1917 (Kamhawi et al. 2000, Volf and Myskova 2007), although other sand fly species have been shown to transmit *L. tropica* in Ethiopia (Gebre-Michael et al. 2004) and northern Israel (Jacobson et al. 2003, Svobodova et al. 2006).

The geographical range of *P. sergenti* is very broad and more widespread than the distribution of *L. tropica*, suggesting some degree of intraspecific variability that may potentially affect the vector competence of different populations of this species (Depaquit et al. 2002). Sequencing of the internal transcribed spacer 2 (ITS2) of 12 populations from 10 countries revealed two principal branches of distinct geographical origin: 1) a more north-east area (Cyprus, Pakistan, Syria, and Turkey) and 2) a more south-west area (Israel, Egypt, Morocco, Sicily; Depaquit et al. 2002). These two branches were confirmed by subsequent studies using random-amplified polymorphic DNA and geometric morphometrics (Dvorak et al. 2006, 2011).

To study the possible consequences of the molecular heterogeneity of *P. sergenti* on the vector competence of *L. tropica*, we established two *P. sergenti* colonies of different geographical origin, one from Turkey (the north-east branch) and the second from Israel (the south-west branch), and experimentally tested their susceptibility to *L. tropica*. As the Turkish colony was naturally infected by the gregarine *Psychodiella sergenti* (Apicomplexa: Eugregarinorida), and the egg-washing procedure by Poinar and Thomas (1984) commonly used to clean gregarines from sand fly colonies is not sufficiently effective in *P. sergenti* (Lantova and Volf 2012), we have now compared the development of *L. tropica* in two groups of Israeli *P. sergenti*, one being infected experimentally by gregarines.

Materials and Methods

Sand Flies and Parasites. Three laboratory colonies of *P. sergenti* were used—1) TU originating from Sanliurfa, Turkey; 2) IS originating from Amnun, Israel; and 3) ISG derived from IS females artificially infected by *Ps. sergenti* as described by Lantova et al. (2010). Sand flies were maintained under standard conditions as previously described by Volf and Volfova (2011). *Leishmania tropica* SU23 (MHOM/TR/98/HM) was maintained at 23°C on M199 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 20% foetal calf serum (Gibco, Life Technologies, Carlsbad, CA), 1% BME vitamins (Sigma-Aldrich), 2% filtered human urine, amikacin (250 mg/ml), and gentamicin (80 mg/ml).

Experimental Infection. Sand fly females (4–7 d old) were fed through a chick-skin membrane on heat-inactivated rabbit blood containing 1×10^6 promastigotes/ml. This infective dose corresponds to <1,000 parasites per female, as bloodmeal volumes taken by

[1] Corresponding author, e-mail: magda.jancarova@seznam.cz.

P. sergenti tested here is not reflected by different susceptibility to *L. tropica*. Current results are consistent with the previously described vector competence of various *P. sergenti* populations for *L. tropica* (Svobodova et al. 2006, Kamhawi 2006, Maroli et al. 2013).

This finding on *P. sergenti* corresponds with results on *Leishmania donovani* vectors: two populations of *Phlebotomus orientalis* Parrot, 1936 from endemic and nonendemic areas in Ethiopia were equally susceptible to *L. donovani*, and the authors (Seblova et al. 2013) concluded that factors other than the vector competence of *P. orientalis* play a role in the epidemiology of *L. donovani* in Ethiopia. Similarly, differences in the distribution of *L. tropica* and its main vector *P. sergenti* may be rather attributed to factors other than the different vector competence of various *P. sergenti* populations.

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