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Letter to the Editors

On monoxenous trypanosomatids from lesions of immunocompetent patients with suspected cutaneous leishmaniasis in Iran

Dear Editors,

We would like to draw the attention of the research community to a recently published paper on identification of monoxenous trypanosomatids of the genus *Crithidia* from lesions of immunocompetent patients with suspected cutaneous leishmaniasis in Iran [1]. We disagree with the authors' interpretation of the data and posit an alternative explanation. Their study does not contain enough information to justify the conclusions they arrived at in their paper.

The important question of whether the insect-dwelling monoxenous (with one host in their life cycle) trypanosomatids can survive in vertebrate hosts has been debated for years. The prevailing opinion in the field is that it can happen only under certain conditions [2–4]. These are: (i) co-infection with another infectious agent that compromises the host immune system, thus allowing the monoxenous trypanosomatid to survive, and (ii) the ability of the monoxenous co-infectant to withstand the elevated temperature of a warm-blooded vertebrate host [5]. In all cases documented thus far, the immunosuppressing agent was either HIV or the dixenous (shuttling between two hosts) *Leishmania donovani* [6–10]. While thermoresistance is an important pre-adaptation for living in warm-blooded vertebrates, it is not sufficient by itself [11, 12]. Sporadic old reports [e.g. 13, 14] were all repudiated over time.

At first glance, the recent account has violated the established paradigm, as it documented the presence of *Crithidia* spp. in lesions of immunocompetent patients [1]. However, the immune status of the patients was not assessed. The authors merely claimed the absence of underlying diseases, which could cause immunodeficiency. In our opinion this is not enough to call such patients immunocompetent.

The bulk of analyses was performed on trypanosomatid cultures and only PCR identification based on gGAPDH gene was done on primary clinical samples (i.e. smears). PCR-based techniques are prone to DNA carry-over, resulting in false positives, and, therefore, cannot be considered as final evidence. The authors used two pairs of custom gGAPDH primers, designed using the sequences of *Leishmania major* and *Crithidia fasciculata*. PCRs with these primers were postulated to identify trypanosomatid genera. However, we checked the specificity of the leishmanial primers and found that they cannot amplify gGAPDH gene from some *Leishmania* spp. (e.g. and importantly, *Leishmania infantum*). Cultures cannot be regarded as representative material for an infection, because the infection may be mixed [15]. From previous studies it is known that cultures from mixed *Leishmania donovani–Leptomonas seymouri* infections contained predominantly or exclusively the monoxenous component [6, 7, 10]. This is apparently due to the different growth rates of these species in laboratory conditions. The same might happen with the material studied here [1].

We suppose that the samples regarded as containing solely *C. fasciculata* originated from a mixed infection by this trypanosomatid with *L. infantum*. This leishmania, typically causing visceral leishmaniasis [16], is known to occasionally produce cutaneous disease [17–19]. Moreover, such cases are known from Iran [20, 21]. The method used by Ghobakhloo *et al.* [1] would not allow detection of *L. infantum* in analysed samples.

Nevertheless, it seems that the authors have discovered a novel thermotolerant monoxenous trypanosomatid closely related to *C. fasciculata*. The appearance of such strains in a tropical/subtropical climate is not surprising, provided constant pressure of the elevated temperature on insects and their parasites [11]. Whether such species are able to infect humans remains to be investigated further with more scrutiny and special attention paid to potential co-infectants.

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