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Novel identification of *Leishmania major* in *Hemiechinus auritus* and molecular detection of this parasite in *Meriones libycus* from an important foci of zoonotic cutaneous leishmaniasis in Iran

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Summary
Background: One of the well-known foci of zoonotic cutaneous leishmaniasis (ZCL) in Iran is Turkemen Sahara, which is located in north eastern Iran. ZCL is a disease of mammals, and humans can become infected as accidental hosts. Many researchers have argued that *Rhombomys opimus* is the only main reservoir host of ZCL in this region of the Golestan province. No other rodents or mammals are thought to host or have been reported to host *Leishmania* parasites in this region. This research was designed and developed to isolate, detect and firmly identify *Leishmania* parasites in mammals and rodents other than *R. opimus*.
Methods: Wild mammals were caught from gerbil burrows. *Leishmania* parasites were detected to assess the infection of reservoir hosts in 2010. Each genomic DNA sample was screened for *Leishmania* infection via nested PCR and sequencing using the internal transcribed spacer ribosomal DNA (ITS-rDNA) identification protocol for parasites.
Results: The greatest number of infections (8/19) were found in *Meriones libycus*. One in three infections was found in *Hemiechinus auritus*, and this is the first report of infection in this species. Only *Leishmania major* was definitively identified and unambiguously typed in *M. libycus* and *H. auritus*. The infection rates in these two wild mammals were not significantly different, and no other gerbil parasites were detected in *M. libycus* or *H. auritus* at our study site.

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Conclusions: Recent findings of *Leishmania turanica* in *R. opimus* and failures to detect *L. turanica* in *M. libycus* may be attributable to unidentified *Leishmania* infections in two *M. libycus* due to unreadable sequences. These cases may represent mixed infections by *L. major* and *L. turanica*. The assumptions that gerbil parasites can be co-infectors provide a starting point for the identification of the causative and potential parasites responsible for the frequent infections that are mainly mediated via sandfly vectors.

Introduction

Turkemen Sahara in northern Iran is one of the most prominent regions for ZCL and is situated on the border of Turkmenistan, which is a prominent part of zoonotic cutaneous leishmaniasis (ZCL) [1]. ZCL is a disease that primarily uses animals such as rodents as reservoir hosts [2]. Humans are an accidental host that can be involved in the transmission cycle of *Leishmania* parasites [3—5]. Geographically, ZCL is widely distributed in Africa, the Middle East, Central Asia, and the Rajasthan area of India [6,7]. ZCL exists in a large variety of types in wild animal hosts and sandfly vectors. *Leishmania* parasites have been isolated from lesions of the ears, noses and eyelids of the large gerbil (*Rhombomys opimus*), the Libyan jird (*Meriones libycus*), the Indian gerbil (*Tatera indica*), the bandicoot rats (*Nesokia indica*), and other rare animals, such as the long-eared hedgehog (*Hemiechinus auritus*) and the long-clawed ground squirrel (*Spermophilopsis leptodactylus*) [8,9]. ZCL is endemic to more than half of the 30 provinces of Iran [10] and is essentially a disease of gerbils that is transmitted by *Phlebotomus papatasi*, *P. caucasicus* and other species of sandflies that breed in gerbil burrows [11,12]. Three different epidemiological types of ZCL have been observed in this country, and four species of rodents served as the principal reservoir hosts in all of the leishmaniasis foci of Iran [1,13,14]. The study of *Leishmania* infection in rodents began in 1953 in north eastern Iran. Although the range of ZCL subsequently expanded to many parts of the country, the isolation and characterization of the parasites have remained unattended [13]. Over the past decade, the annual incidence of ZCL has gradually risen due to increasing sandfly — human contact and agricultural development in many areas of the world endemic for leishmaniasis, including Iran, leading to greater exposure of people to sandfly bites and *Leishmania* parasite infection [15,16].

Some publications have reported that *R. opimus*, a member of the rodent family of Gerbilidae, acts as the main reservoir host of ZCL in the Turkemen Sahara region [1,2]. In this investigation, we found that *M. libycus*, the long-eared hedgehog *H. auritus*, and *R. opimus* carried ZCL. Notably, the impression smears from the ears of each rodent were obtained by scratching, and this method has previously been published and used in studies conducted in central Iran and the mentioned districts [2,10,14]. Based on our experiences, we selected the rodent ear scratch method for the current study. In our previous study that was conducted in the same location, a large number of *R. opimus* were examined for *Leishmania* infections. In total, 74 of the 227 *R. opimus* examined had *Leishmania* infections (59 with *Leishmania major*, 6 with *Leishmania turanica* and 9 in which the *Leishmania* species was not identified). Currently, there are no reports of *Leishmania* infection in *M. libycus* or *H. auritus* in the Turkemen Sahara region [2,10]. Accordingly, PCR assay and ITS-rDNA gene sequencing, along with three conventional methods, were employed to directly detect and/or identify the *Leishmania* species infecting captured wild mammals. The samples were screened for *Leishmania* infections using specimens that were collected in 2009—2010 from gerbil burrows and nearby villages in the Golestan province (Turkemen Sahara, North East of Iran) (Fig. 1).

Materials and methods

Study sites and collections and identification of wild mammals

The study sites were determined based on reports from the local health authorities about outbreaks of human ZCL infections (unpublished data and unofficial reports). The active colonies of rodents were identified, and the rodents were trapped alive with wooden and wire traps in various parts of these areas (Fig. 1). The specimens were collected from colonies of gerbil burrows located around the villages in which ZCL was endemic