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Borrelia persica infection in rock hyraxes

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ABSTRACT

Tick-borne relapsing fever (TBRF) is an acute infectious disease caused by arthropod-borne spirochetes of the genus *Borrelia* and characterized by recurrent episodes of fever. *Borrelia persica*, the causative agent of this disease in Israel, is transmitted by the argasid tick *Ornithodoros tholozani*. There is little information about the maintenance and possible vertebrate reservoirs of *B. persica* in nature, but the tick *O. tholozani* is known to feed on animals that enter its habitat in caves, rock crevices and shady environments. The rock hyrax (*Procavia capensis*) is commonly found in such habitats and may therefore serve as a reservoir host for *O. tholozani*.

Blood and spleen samples from rock hyraxes were collected from twelve locations in Israel and the West Bank during 2009–2014 to test if these animals may be infected with *B. persica*. Real-time PCR targeting a segment of the flagellin (*flaB*) gene was initially used to detect *B. persica*. Positive samples were further analyzed by PCR, using a segment of the glycerophosphodiester phosphodiesterase (*GlpQ*) gene for additional confirmation. *Borrelia* species were identified by nucleotide sequence analysis and the copy number of *Borrelia* was quantified in blood and spleen samples based on the number of *Borrelia* 16S rRNA gene copies.

A total of 112 hyraxes were examined, with both blood and spleen samples tested from 108 animals. Nine hyraxes were infected with *B. persica*, with a prevalence of 8%. Of these, two animals were positive for both blood and spleen samples, three only for blood and four only for the spleen. The number of DNA copies of *Borrelia* 16S rRNA was significantly higher in blood (5×10^6 to 9.2×10^8 /ml blood) compared to spleen (2.1×10^4 to 1.0×10^6 /ml).

We conclude that rock hyraxes are possible reservoirs for *B. persica* because they have long lifespans and gregarious habits, share habitats with vector ticks, and are naturally infected with this spirochete. Further studies should be conducted in the future to evaluate the competence of hyraxes as reservoirs for *B. persica* infection.

1. Introduction

Tick-borne relapsing fever (TBRF) is an acute infectious disease characterized by recurring episodes of fever in humans (Barbour and Hayes, 1986). The disease is caused by *Borrelia* spirochetes, transmitted to humans by the bites of infected argasid ticks. TBRF *Borrelia* species are considered to be specific to their tick vectors (Parola and Raoult, 2001). *Borrelia persica* causes TBRF in Israel and other countries in the Near East, and is transmitted by the tick *Ornithodoros tholozani* (Avivi et al., 1973, Parola and Raoult, 2001). The global distribution area of *O. tholozani* overlaps the distribution range of TBRF caused by *B. persica* (Assous and Wilamowski, 2009).

Ornithodoros tholozani has a wide host range, including man, sheep, goat, camels, cattle, porcupines, hedgehogs, foxes, jackals and rodents (Hoogstraal, 1985). This tick is commonly encountered in caves, ruins,

animal shelters and burrows, and in the northern part of its distribution (Southern Russia, Iran, Northern India and Afghanistan) it can also be found in stables, barns, clay and stone fences, storerooms and human dwellings (Avivi et al., 1973; Hoogstraal, 1985).

Ornithodoros tholozani ticks and rock hyraxes (*Procavia capensis*) are both prevalent in similar ecological niches in Israel. Rock hyraxes have a life span of 4–9 years and live in groups of up to 50 individuals (Ashford, 1996; Barocas et al., 2011). Their populations in Israel are expanding and encroaching human settlements, where they live in rock crevices, within boulder mounds created during land clearing for housing construction, or in caves (Jacobson et al., 2003; Svobodova et al., 2006; Kershenbaum et al., 2011). In these environments, they can share the same habitat and be in close contact with *O. tholozani* ticks that prefer caves and shady environments (Avivi et al., 1973), and thus may provide blood meals to the ticks and potentially become infected

Abbreviations: TBRF, tick-borne relapsing fever; *GlpQ*, glycerophosphodiester phosphodiesterase gene; *flaB*, flagellin gene

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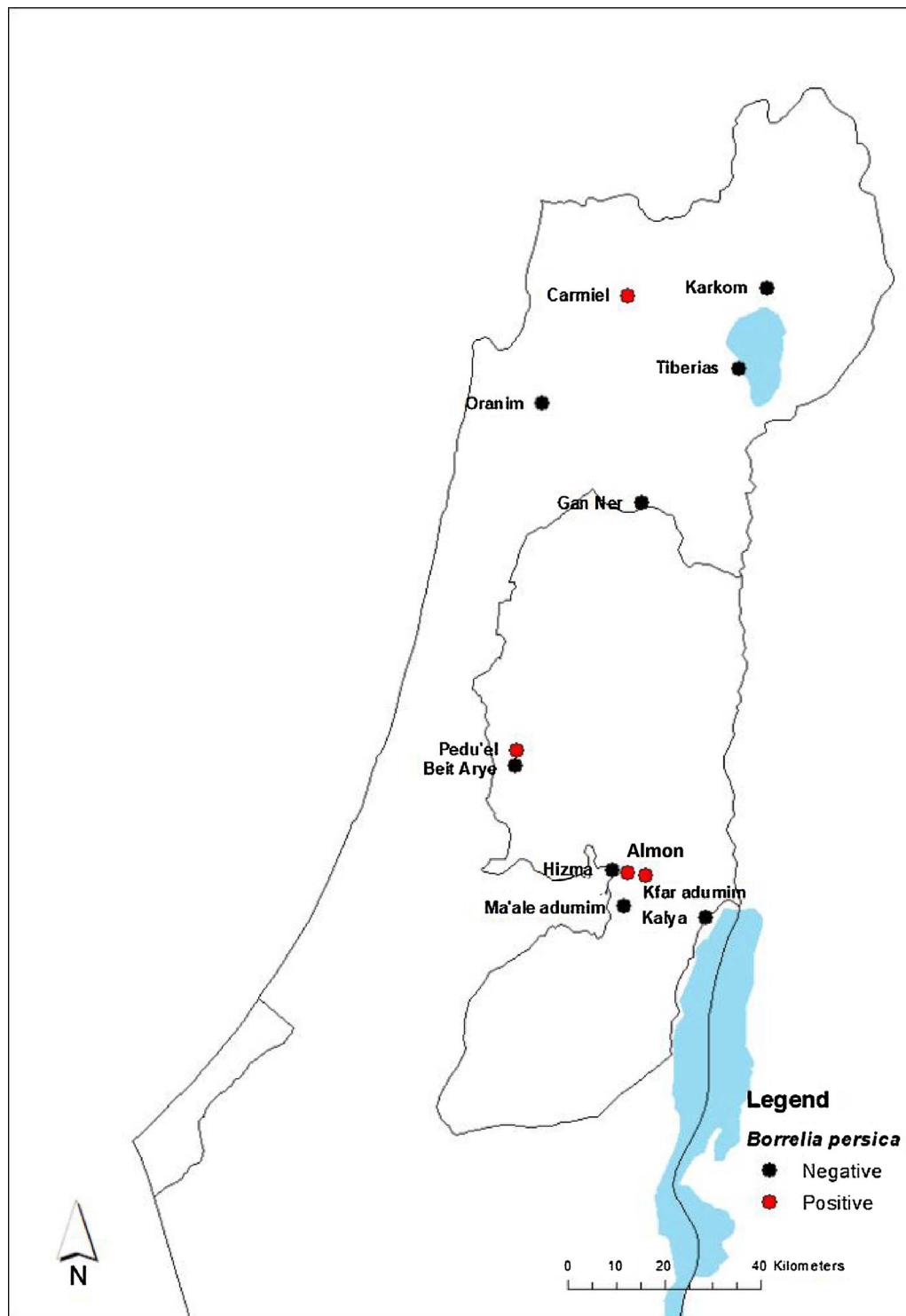


Fig. 1. Locations where hyraxes were collected indicating the presence or absence of infection with *B. persica*.

with *B. persica*.

The main purpose of this study was to evaluate the prevalence of infection with *B. persica* in hyraxes living in proximity to human habitations in Israel, and to estimate the bacteremia of infected animals.

2. Materials and methods

2.1. Sample collection and DNA extraction

Hyrax samples were collected from twelve locations in Israel and the West Bank during 2009–2014 by the Israeli Nature and Parks

Authority as part of an epidemiological study on infection with *Leishmania tropica* and *Leishmania major* in wild animals. Trapping was carried out in the following locations: Almon, Beit Arye, Carmiel, Gan Ner, Hizma, Kalya, Karkom, Kfar Adumim, Ma'ale Adumim, Oranim, Pedu'el and Tiberias (Fig. 1). Hyraxes were trapped using Tomahawk live box traps (Tomahawk traps, Hazelhurst, Wisconsin, U.S.A.) placed in secure shady spots, and baited with leaves and vegetables. Trapped hyraxes were anesthetized by intra-muscular injection of ketamine-HCl (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, U.S.A.) at 10 mg/kg. Blood was collected in EDTA tubes for DNA extraction and kept at -20°C for further analysis. Animals were euthanized by intravenous

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