



Transmission of the Human Relapsing Fever Spirochete *Borrelia persica* by the Argasid Tick *Ornithodoros tholozani* Involves Blood Meals from Wildlife Animal Reservoirs and Mainly Transstadial Transfer

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ABSTRACT *Borrelia persica*, transmitted by the argasid tick *Ornithodoros tholozani*, causes human tick-borne relapsing fever in the Middle East and Central Asia. Infection is acquired often when visiting tick-infested caves and reported to be transmitted mainly transovarially between ticks, occasionally infecting humans. To study the epidemiology of this infection, ticks were trapped in 24 caves in 12 geographic zones covering all of Israel and identified morphologically. DNA was extracted from larvae, nymphs, and adult stages from each location and PCR followed by DNA sequencing was performed to identify *Borrelia* infection, tick species, and tick blood meal sources. We collected 51,472 argasid ticks from 16 of 24 caves surveyed. We analyzed 2,774 *O. tholozani* ticks, and 72 (2.6%) from nine caves were PCR positive for *B. persica*. Infection rates in male, female, and nymphal ticks (4.4%, 3%, and 3.2%, respectively) were higher than in larva ($P < 0.001$), with only 3 (0.04%) positive larvae. Presence of blood meal was associated with *B. persica* infection in ticks ($P = 0.003$), and blood meals of golden jackals, red foxes, and Cairo spiny mouse were associated with infection ($P \leq 0.043$). PCR survey of 402 wild mammals revealed *B. persica* infection with the highest rates in social voles (22%), red foxes (16%), golden jackals (8%), and Cairo spiny mice (3%). In conclusion, although transovarial tick transmission of *B. persica* occurs at low levels, ticks apparently acquire infection mainly from wildlife canid and rodents and may eventually transmit relapsing fever borreliosis to humans who enter their habitat.

IMPORTANCE *Borrelia persica* is a spirochete that causes tick-borne relapsing fever in humans in an area that spans from India to the Mediterranean. Until now, it was thought that the soft tick vector of this infection, *Ornithodoros tholozani*, is also its main reservoir and it transmits *B. persica* mostly transovarially between tick generations. This study showed that tick infection with *B. persica* is associated with feeding blood from wild jackals, foxes, and rodents and that transovarial transmission is minimal. Since *O. tholozani* ticks are found in isolated caves and ruins, it is assumed that wild canids who migrate over long distances have a major role in the transmission of *B. persica* between remote tick populations, and it is then maintained locally also by rodents and eventually transferred to humans during tick bites. Prevention of human infection could be achieved by restricting entrance of canines and humans to habitats with *O. tholozani* populations.

KEYWORDS *Ornithodoros tholozani*, *Borrelia persica*, relapsing fever, Argasidae, blood meal

Tick-borne relapsing fever (TBRF) is transmitted to humans by the bite of infected argasid ticks (1). TBRF was reported initially in Israel under the British Mandate in 1919 in British troops and subsequently studied by Adler, who made a detailed clinical

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description of the disease and pointed out that visiting caves was a common element in the medical history of infected patients (2, 3). TRBF caused by *Borrelia persica* and transmitted by the tick *Ornithodoros tholozani* is common in Israel and other countries in the Near East, extending from India and Central Asia to Egypt (4, 5). The disease is characterized by spirochetemia with episodes of fever, separated by afebrile intervals. Complications with fatal consequences include neurological manifestations, liver pathology, and cardiac dysfunction (5–7). Relapsing fever is a notifiable disease in humans in Israel, and its incidence was reported to be 0.11/100,000 population among civilians and 6.4/100,000 population among military personnel (8); however, the disease is probably underreported, as *B. persica* is susceptible to many types of antibiotics, and patients with high fever are often treated before diagnosis is complete (9). From 2004 to 2018, 92 patients, including 30 children, were diagnosed with relapsing fever caused by *B. persica* in two hospitals in Jerusalem (10), and 47 patients were diagnosed with this infection in a single Jerusalem hospital between 2009 and 2019 (11). The transmission of *B. persica* in *O. tholozani* has been reported to be transovarial, as described also for other TBRF *Borrelia* spp., and it has been suggested that the tick itself may act as a reservoir for infection, passing it on between generations of ticks and occasionally transmitting it to humans (12, 13). Rodents have been considered the main reservoirs of TBRF caused by other relapsing fever borreliae, such as *Borrelia hermsii*, *Borrelia parkeri*, and *Borrelia turicatae* in North America and *Borrelia crocidurae* and *Borrelia hispanica* in Africa and Spain (14–17). The presence of an animal reservoir for *B. persica* has not been demonstrated previously, and although experimental infection was described in laboratory mice and guinea pigs (18, 19), no records of natural rodent infection have been published. We have recently described infection and clinical disease with *B. persica* in domestic dogs and cats and infection in wild rock hyraxes (*Procapra capensis*) from Israel (20, 21). Natural infection in rock hyraxes constituted the only report of *B. persica* in wild animals in Israel. *Ornithodoros tholozani* usually takes a short blood meal of 15 to 60 min (22), and it is not found attached to animals in tick surveys (4). Its ecological niche is typically in shady, moist, and relatively cool environments such as caves, ruins, and rock crevices (4).

The study of arthropod blood meals is a useful method that can help elucidate the identity of their hosts and understand transmission cycles of vector-borne pathogens (23). Mitochondrial genes have been used as reliable targets for identification of vertebrate blood meal sources of hematophagous arthropods, including ticks, due to the variation in their nucleotide sequences between vertebrate host species and the high number of mitochondrial gene copies found per cell, which increases the likelihood of detecting host DNA in the vector's blood meal (23–27).

In order to better understand the epidemiology and transmission of TBRF caused by *B. persica*, we planned a study to assess the presence and identity of soft ticks in caves in Israel, detect infection with *Borrelia* spp. in these ticks, assess the presence and source of blood meals in ticks at various life stages, survey wild mammals such as those living near locations of infected tick hosts for infection with relapsing-fever *Borrelia* spp., and determine possible associations between tick and wildlife infection.

RESULTS

Ticks. Altogether, 51,472 argasid ticks were collected from November 2013 to August 2015 from 16 out of 24 (67%) cave sites surveyed in Israel (Table 1; Fig. 1). The total number of ticks trapped in each cave where ticks were found varied from 1 tick (cave 10) to 26,782 (cave 5), with a median of 181. We analyzed 2,777 ticks (nymphs, $n = 1,036$; larvae, $n = 787$; adult males, $n = 525$; and adult females, $n = 429$) from 16 caves.

Tick infection by *Borrelia* spp. *flaB* DNA segments of 270 to 273 bp with 100% identity to *B. persica* sequences available in GenBank were amplified from 72 ticks (13 females, 23 males, 33 nymphs, and 3 larvae) out of a total of 2,777 ticks analyzed. Infection was detected in 9 of the 16 caves (56%) where ticks were found (Table 1; Fig. 1). The prevalence of *B. persica* infection among ticks in each cave ranged from 0.6 to

TABLE 1 Locations and number of argasid ticks collected in caves in Israel from November 2013 to August 2015

| Cave no. | Cave name | Cave coordinates | No. of female ticks (no. of positive ticks) | No. of male ticks (no. of positive ticks) | No. of nymph ticks (no. of positive ticks) | No. of larval ticks (no. of positive ticks) | Total no. of ticks (no. of positive ticks) |
|----------|------------------|------------------------------|---|---|--|---|--|
| 1 | Kerem Ben Zimra | 33°01'58.93"N, 35°27'12.08"E | 1 (0) | 1 (0) | 1 (0) | 52 (0) | 55 (0) |
| 2 | Lavi | 32°46'35.85"N, 35°25'50.94"E | 7 (0) | 3 (0) | 103 (2) | 0 | 115 (2) |
| 3 | Shh'in 1 | 32°46'06.30"N, 35°16'32.37"E | 1,208 (1) | 1,507 (0) | 12,568 (3) | 3,201 (1) | 18,484 (5) |
| 4 | Shh'in 2 | 32°45'47.35"N, 35°16'18.28"E | 25 (1) | 12 (1) | 123 (9) | 5 (1) | 165 (12) |
| 5 | Nurit | 32°32'04.62"N, 35°21'46.32"E | 306 (5) | 144 (5) | 4,989 (6) | 21,343 (0) | 26,782 (16) |
| 6 | Avinadav | 32°27'14.32"N, 35°26'04.39"E | 69 (3) | 127 (12) | 1,010 (2) | 139 (0) | 1,345 (17) |
| 7 | Herzeliya | 32°10'04.12"N, 34°48'50.73"E | 0 | 0 | 0 | 0 | 0 |
| 8 | Afeka | 32°07'46.56"N, 34°48'32.88"E | 0 | 0 | 0 | 0 | 0 |
| 9 | Ras el-Amud | 31°44'08.95"N, 35°03'47.74"E | 0 | 0 | 0 | 0 | 0 |
| 10 | Emek Hamatzlevah | 31°46'18.31"N, 35°12'22.14"E | 0 | 0 | 1 (0) | 0 | 1 (0) |
| 11 | Beit Guvrin 1 | 31°35'52.28"N, 34°53'27.47"E | 3 (0) | 5 (0) | 42 (0) | 315 (0) | 365 (0) |
| 12 | Beit Guvrin 2 | 31°36'2.88"N, 34°53'26.29"E | 22 (0) | 31 (1) | 156 (5) | 178 (0) | 387 (6) |
| 13 | Gvaram | 31°34'50.28"N, 34°36'35.85"E | 1 (0) | 1 (0) | 19 (0) | 0 | 21 (0) |
| 14 | Ruchama | 31°30'08.66"N, 34°41'12.75"E | 0 | 1 (0) | 3 (0) | 0 | 4 (0) |
| 15 | Yatir | 31°20'03.01"N, 35°02'09.06"E | 0 | 2 (0) | 30 (0) | 0 | 32 (0) |
| 16 | Arad 1 | 31°16'08.33"N, 35°13'51.04"E | 13 (0) | 21 (0) | 125 (1) | 35 | 196 (1) |
| 17 | Arad 2 | 31°14'37.21"N, 35°13'13.51"E | 0 | 0 | 0 | 0 | 0 |
| 18 | Dimona | 31°07'26.29"N, 35°07'20.44"E | 0 | 1 (0) | 38 (0) | 0 | 39 (0) |
| 19 | Ashalim | 30°56'37.07"N, 34°44'20.76"E | 28 (1) | 42 (0) | 531 (0) | 96 (1) | 697 (2) |
| 20 | Nitzana | 30°51'50.41"N, 34°25'40.20"E | 67 (2) | 127 (4) | 2,111 (5) | 479 (0) | 2,784 (11) |
| 21 | Ovdat | 30°47'35.47"N, 34°46'23.29"E | 0 | 0 | 0 | 0 | 0 |
| 22 | Nafha | 30°41'55.36"N, 34°47'08.17"E | 0 | 0 | 0 | 0 | 0 |
| 23 | Timna | 29°47'27.15"N, 34°55'58.64"E | 0 | 0 | 0 | 0 | 0 |
| 24 | Eilat | 29°30'27.60"N, 34°55'06.91"E | 0 | 0 | 0 | 0 | 0 |
| Total | | | 1,753 (13) | 2,025 (23) | 21,847 (33) | 25,810 (3) | 51,472 (72) |

8.6% (standard deviation [SD], 2.1). There was no significant difference between the infection rates in males, females, and nymphs (4.4%, 3%, and 3.2%, respectively; chi-square $P=0.4$); however, the infection rate in larvae was significantly lower, with only 3 positive larvae (2 individuals and 1 pool) out of 787 analyzed (0.04%; chi-square $P<0.001$). The pools included 5 larvae each, and due to the low percentage of infection found in single larvae (2/391; 0.5%) and considering the minimal infection rate (MIR), calculated according to the ratio of positive pools to the total number of larvae analyzed (28), the positive pool was considered to stem from one infected larva.

The 270- to 273-bp *flaB* DNA segments of *B. persica* amplified from ticks were 98 to 100% identical to each other and clustered together in a phylogenetic analysis with *B. persica* from a tick and 2 human patients from Israel (GenBank accession numbers [DQ679905.1](#), [DQ679906.1](#), and [DQ679907](#), respectively) (Fig. 2). In addition, 19 ticks, from which 270- to 273-bp-long *B. persica flaB* sequences were amplified (29), were positive also by PCR for an overlapping 720-bp segment of *flaB* (30), and 14 707- to 710-bp-long *flaB* sequences from ticks in the current study were deposited in GenBank (accession numbers [KX258784](#) to [KX258797](#)). Of the 72 ticks positive for *B. persica flaB*, 67 were also positive by the *glpQ* PCR (31). Analysis of the *glpQ* sequences revealed that they all clustered together with *B. persica* sequences from a tick and a human from Israel (GenBank accession numbers [HM161656](#) and [HM161657](#), respectively) (Fig. S1 in the supplemental material). Three *glpQ* sequences from ticks in this study were deposited in GenBank ([MH923346](#) to [MH923348](#)). In addition, one tick from cave 5 harbored DNA that was 100% identical (*flaB* gene) to *Borrelia* sp. strain ZFB15_113, designated "*Candidatus Borrelia fainii*" (GenBank accession no. [LC375836.1](#)), a spirochete found to infect a febrile human patient in Zambia and transmitted by the soft tick *Ornithodoros faini*, which also infests wild bats (*Rousettus aegyptiacus*) (32) (Fig. 2). This sequence was deposited in GenBank as accession number [MK636871](#).

Of the nine caves where infected ticks were found (Fig. 1), four were sites where human cases of TBRF were reported to be acquired (cave no. 2, 12, 16, and 19), an

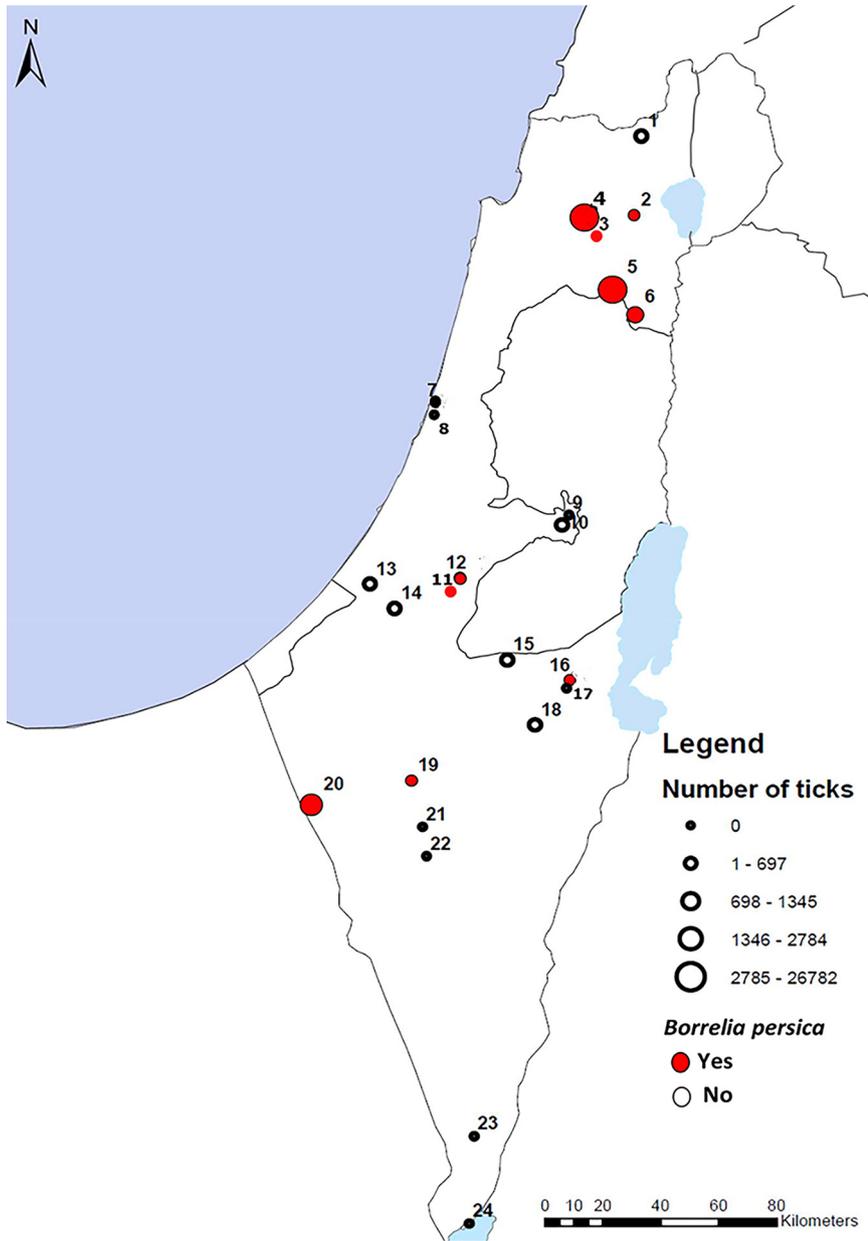


FIG 1 Map showing the tick collection sites represented with circles, where red circles symbolize sites where *B. persica*-infected ticks were found and circle size represents the number of ticks collected in each site. Numbers in the map represent the cave identity number.

additional four were suspected of being infected (no. 3, 4, 5, and 6), and one (no. 20) was from a location where human infection was not reported before. Of the six caves where no infection was found in ticks, one cave (no. 10) with one tick was located in an area with a TBRF report, two were caves suspected of infection (no. 11 and 18), and the other four were from locations where human infection was not reported before (no. 1, 13, 14, and 15). Of the nine caves where no ticks were trapped, one was a cave located in an area from which cases of TBRF have been reported (no. 21), and seven were sites from which no report of previous human infection is known (no. 7, 8, 9, 17, 22, 23, and 24).

Tick blood meal identification. Vertebrate blood meals were found in 1,415 ticks out of the 2,777 analyzed (51%). Blood meals were detected in all tick life stages (females, $n = 355$; males, $n = 422$; nymphs, $n = 622$; and larvae, $n = 16$) from all the caves

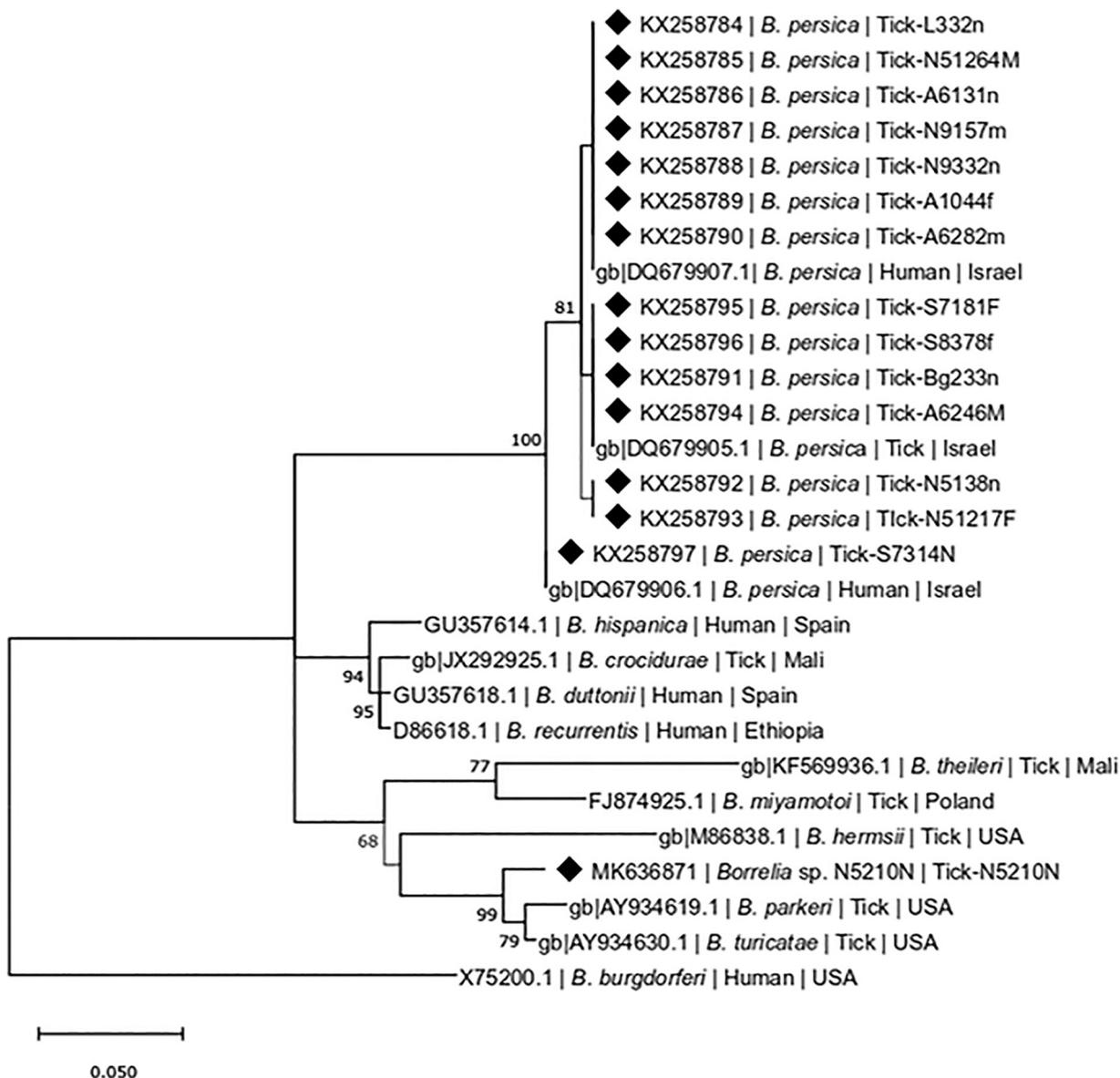


FIG 2 Maximum-likelihood phylogram comparing 270- to 273-bp DNA sequences of the *flaB* gene from *B. persica* detected in *O. tholozani* ticks included in the study to sequences corresponding to other *B. persica* GenBank accession numbers and from other *Borrelia* spp.; *B. burgdorferi* (GenBank accession no. X75200.1) was used as outgroup. The Tamura 3-parameter model was chosen with a pairwise deletion procedure. Numbers at nodes correspond to a percentage confidence level higher than 70% in a bootstrap test performed on 1,000 replicates. New sequences derived from this study are marked with black diamond squares and deposited in GenBank. The scale bar corresponds to a distance of 0.05 nucleotide substitution per site.

analyzed where ticks were found, with the exception of two caves, no. 10, where only a single tick was collected and analyzed, and no. 14, where two ticks were analyzed and none were engorged (Table 1). The prevalence of adult females and males harboring blood meals was significantly higher than nymphs and larvae (82.8%, 80.2%, 60%, and 2%, respectively; chi-square $P < 0.0001$). The prevalence of ticks carrying a blood meal varied significantly between the different caves (range, 6 to 76.7%; chi-square $P < 0.001$), with the highest prevalence in caves 15 and 20 (76.7 and 66.5%, respectively) and the lowest in caves 1 and 11 (6 and 9%, respectively).

DNA of 25 different vertebrate species was detected in ticks (Table 2). The most prevalent blood meal found was from the Indian crested porcupine (*Hystrix indica*) (71.5%), followed by the rock hyrax (*Procapra capensis*), the European badger (*Meles meles*) (6.8% and 4.3%, respectively), and 22 other species (Table 2). In addition, mixed

TABLE 2 Identified animal sources of blood meal from ticks according to life stage and number of ticks infected with *B. persica* in each stage^a

| Source of blood meal (Latin species name) | Total no. of ticks | No. of adult male ticks (no. of infected ticks) | No. of adult female ticks (no. of infected ticks) | No. of nymphs (no. of infected ticks) | No. of larvae (no. of infected ticks) |
|---|--------------------|---|---|---------------------------------------|---------------------------------------|
| Single blood meal source | | | | | |
| Indian crested porcupine (<i>Hystrix indica</i>) | 1,088 | 347 (8) | 291 (5) | 450 (11) | 0 |
| Rock hyrax (<i>Procapra capensis</i>) | 103 | 27 (1) | 25 (1) | 50 (1) | 1 |
| European badger (<i>Meles meles</i>) | 66 | 18 | 16 | 30 (1) | 2 |
| Wild goat (<i>Capra aegagrus</i>) | 41 | 5 | 7 | 29 (1) | 0 |
| Red fox (<i>Vulpes vulpes</i>) | 48 | 23 (5) | 20 (2) | 5 | 0 |
| Human (<i>Homo sapiens</i>) | 39 | 7 | 1 | 24 | 7 |
| Black rat (<i>Rattus rattus</i>) | 19 | 5 | 1 | 13 (1) | 0 |
| Golden jackal (<i>Canis aureus</i>) | 17 | 5 (2) | 2 (1) | 10 (2) | 0 |
| Cairo spiny mouse (<i>Acomys cahirinus</i>) | 10 | 5 (2) | 3 | 1 | 1 |
| Domestic cat (<i>Felis catus</i>) | 7 | 1 | 1 | 5 | 0 |
| Sheep (<i>Ovis aries</i>) | 5 | 1 | 0 | 4 | 0 |
| Domestic dog (<i>Canis lupus familiaris</i>) | 5 | 2 | 0 | 0 | 3 |
| Turkey (<i>Meleagris gallopavo</i>) | 4 | 0 | 0 | 3 | 1 |
| Cow (<i>Bos taurus</i>) | 3 | 0 | 1 | 2 | 0 |
| Striped hyena (<i>Hyaena hyaena</i>) | 3 | 0 | 0 | 3 | 0 |
| Mongoose (<i>Herpestes</i> sp.) ^b | 3 | 0 | 0 | 3 (1) | 0 |
| Egyptian fruit bat (<i>Rousettus aegyptiacus</i>) | 3 | 1 | 0 | 2 | 0 |
| Wild boar (<i>Sus scrofa</i>) | 2 | 0 | 0 | 1 | 1 |
| Caracal (<i>Caracal caracal</i>) | 1 | 0 | 0 | 1 | 0 |
| Macedonian mouse (<i>Mus macedonicus</i>) | 1 | 0 | 0 | 1 | 0 |
| Leschenault's rousette (<i>Rousettus leschenaultii</i>) | 1 | 0 | 1 | 0 | 0 |
| Mouse (<i>Mus musculus</i>) | 1 | 0 | 0 | 1 | 0 |
| Yellow-necked mouse (<i>Apodemus flavicollis</i>) | 1 | 0 | 0 | 1 | 0 |
| Etruscan shrew (<i>Suncus etruscus</i>) | 1 | 0 | 1 | 0 | 0 |
| Pigeon (<i>Columba livia</i>) | 1 | 0 | 0 | 1 | 0 |
| Mixed blood meals in the same tick | | | | | |
| Indian crested porcupine + rock hyrax | 18 | 7 (2) | 4 | 7 | 0 |
| Indian crested porcupine + rock hyrax + red fox | 13 | 8 (2) | 4 | 1 | 0 |
| Indian crested porcupine + European badger | 4 | 1 | 1 | 2 (1) | 0 |
| Indian crested porcupine + red fox | 3 | 1 | 2 | 0 | 0 |
| Human + European badger | 1 | 0 | 0 | 1 | 0 |
| Wild goat + human | 2 | 0 | 0 | 2 | 0 |
| Indian crested porcupine + human | 5 | 0 | 0 | 5 | 0 |
| Turkey + Indian crested porcupine | 1 | 0 | 0 | 1 | 0 |
| Wild goat + Indian crested porcupine | 2 | 0 | 0 | 2 | 0 |

^aIdentification of blood meals was based on PCR and sequencing. Amplicons of more than 200 base pairs and successful identification of the host with >98% identity of first hit by BLAST compared to sequences available in GenBank were obtained.

^b95% identity to *Herpestes javanicus* was obtained.

blood meals with more than one blood source were found in 53 ticks (3.7% of all ticks with blood meals) (Table 2). The Indian porcupine was the most abundant host blood meal source in male, female, and nymphal ticks (77.6, 78.6, and 70%, respectively); however, in larvae, the most abundant blood meal was from humans (*Homo sapiens*) (43.8%), followed by the domestic dog (*Canis lupus familiaris*) (18.7%). The presence of rock hyrax blood was the second most prevalent blood meal in males, females, and nymphs (6, 6.7, and 7.8%, respectively).

***Borrelia* sp. infection and blood meals in ticks.** Of the 72 *B. persica*-infected ticks, 50 were positive for the presence of a blood meal. The rate of infected ticks with blood meal was significantly higher than those infected without blood meal (68% and 32%, respectively; chi-square $P=0.003$). The rates of infected ticks with blood meals according to their life stage were 95.6%, 69.2%, 54.5%, and 0%, for males, females, nymphs, and larvae, respectively. The rate of infection among engorged ticks was significantly higher in males compared to nymphs and larvae (likelihood ratio chi-square $P=0.002$ and $P=0.001$, respectively). No significant differences in infection rates were observed between engorged males and females and between engorged females, nymphs, and larvae.

TABLE 3 Infected ticks according to the animal sources of blood meal and life stage

| Source of blood meal (Latin species name) | Total no. of infected ticks (%) ^a | No. of infected adult male ticks (%) | No. of infected adult female ticks (%) | No. of infected nymphs (%) | No. of infected larvae (%) |
|--|--|--------------------------------------|--|----------------------------|----------------------------|
| Single blood meal source | | | | | |
| Indian crested porcupine (<i>Hystrix indica</i>) | 24 (2.2) | 8 (2.3) | 5 (1.7) | 11 (2.4) | 0 |
| Rock hyrax (<i>Procapra capensis</i>) | 3 (4) | 1 (3.7) | 1 (4) | 1 (2) | 0 |
| European badger (<i>Meles meles</i>) | 1 (2.7) | 0 | 0 | 1 (3.3) | 0 |
| Wild goat (<i>Capra aegagrus</i>) | 1 (2.7) | 0 | 0 | 1 (3.4) | 0 |
| Red fox (<i>Vulpes vulpes</i>) | 7 (22) ^b | 5 (21.7) | 2 (10) | 0 | 0 |
| Black rat (<i>Rattus rattus</i>) | 1 (5.5) | 0 | 0 | 1 (7.7) | 0 |
| Golden jackal (<i>Canis aureus</i>) | 5 (29) ^b | 2 (40) | 1 (50) | 2 (20) | 0 |
| Cairo spiny mouse (<i>Acomys cahirinus</i>) | 2 (20) ^c | 2 (40) | 0 | 0 | 0 |
| Mongoose (<i>Herpestes</i> sp.) | 1 (33.3) | 0 | 0 | 1 (33.3) | 0 |
| Mixed blood meals | | | | | |
| Indian crested porcupine + rock hyrax | 2 (11.1) | 2 (28.6) | 0 | 0 | 0 |
| Indian crested porcupine + rock hyrax + red fox | 2 (15.3) | 2 (25) | 0 | 0 | 0 |
| Indian crested porcupine + European badger | 1 (25) | 0 | 0 | 1 (50) | 0 |

^aTo calculate the percentage of infection, only animals with one blood meal source were evaluated.

^bStatistical significance; $P < 0.001$.

^cStatistical significance; $P = 0.043$.

The blood meal sources identified in the 50 *B. persica*-infected ticks included the Indian crested porcupine ($n = 24$; 2.3% of the total ticks with *Hystrix indica* blood meal), red fox (*Vulpes vulpes*) ($n = 7$; 22.6%), golden jackal (*Canis aureus*) ($n = 5$; 29.4%), rock hyrax (*P. capensis*) ($n = 3$; 4.3%), Cairo spiny mice (*Acomys cahirinus*) ($n = 2$; 20%), and one infected tick with blood from each of the following species: mongoose (*Herpestes* sp.) (33%), European badger (*Meles meles*) (3%), goat (*Capra aegagrus*) (2.4%), and black rat (*Rattus rattus*) (5.3%) (Table 3). There were also infected ticks with blood meals from two animals, i.e., porcupine and hyrax ($n = 2$) and badger and porcupine ($n = 1$), and 2 ticks with infected blood from three animal species, i.e., porcupine, hyrax, and red fox.

Infected tick blood meals from the red fox and golden jackal were significantly more frequent than the rest of the animal blood meal sources in infected ticks with a single blood meal (chi-square and Fisher's test, $P < 0.001$). Blood meals from the Cairo spiny mouse were also more frequent, with a more moderate level of significance (Fisher's test, $P = 0.043$). Porcupine blood meal was significantly less frequent than other sources of blood meal in *B. persica*-infected ticks (chi-square $P = 0.008$).

Tick sequence analysis. We analyzed 216 ticks using the mitochondrial 16S rRNA gene PCR, which produced DNA sequences of 408 bp. Molecular alignment revealed an identity of 98.3 to 99.0% with sequences of *O. tholozani* present in GenBank (accession numbers [NC039830.1](#) and [MF818023.1](#)), which were also the first match by BLAST analysis. Eight tick 16S rRNA gene sequences from *O. tholozani* ticks were deposited in GenBank as accession numbers [MF615990](#) to [MF615997](#), and the tick's genetic variability was analyzed (Fig. S2).

***Borrelia* sp. infection in animals.** The study included 402 animals belonging to 13 species and 4 mammalian families (Table 4). In total, 459 samples were analyzed (402 blood and 57 spleen samples). Samples of rodents and insectivores were collected from 11 sites, and red fox and golden jackal samples were obtained from 23 and 45 sites, respectively (Fig. 3). Twenty-eight animals were positive for *B. persica* infection by PCR, comprising 7% of the animals tested. These included 13 social voles (22.4% of the total number of social voles), 5 red foxes (15.6%), 5 golden jackals (8%), 4 Cairo spiny mouse (3%), and one fat sand rat (8.3%) (Table 4).

Thirty-four samples were positive for *B. persica* by PCR, including 25 blood and 9 spleen samples. Six social voles were positive both in blood and spleen, 4 only in blood, and 3 only in the spleen (Table 4). All *flaB* sequences were 99% to 100% identical to *B. persica* sequences available in GenBank and clustered in a phylogram separately from other TBRF *Borrelia* spp. (Fig. S3).

TABLE 4 Animal species sampled, type of sample (blood or spleen), and infection with *B. persica*

| Family | Species | No. of animals tested | No. of <i>B. persica</i> -infected animals | No. of positive blood samples/total no. of total blood samples | No. of positive spleen samples/total no. of spleen samples | % infected with <i>B. persica</i> |
|---|--|-----------------------|--|--|--|-----------------------------------|
| Cricetidae | Social vole (<i>Microtus socialis</i>) | 58 | 13 | 10/58 | 9/57 | 22.4 |
| Muridae | Black rat (<i>Rattus rattus</i>) | 1 | 0 | 0 | 0 | 0 |
| | Cairo spiny mouse (<i>Acomys cahirinus</i>) | 133 | 4 | 4/133 | 0 | 3.0 |
| | Tristram's jird (<i>Meriones tristrami</i>) | 48 | 0 | 0 | 0 | 0 |
| | House mouse (<i>Mus musculus</i>) | 43 | 0 | 0 | 0 | 0 |
| | Indian crested porcupine (<i>Hystrix indica</i>) | 2 | 0 | 0 | 0 | 0 |
| | Eastern broad-toothed field mouse (<i>Apodemus mystacinus</i>) | 3 | 0 | 0 | 0 | 0 |
| | Fat sand rat (<i>Psammomys obesus</i>) | 12 | 1 | 1/12 | 0 | 8.3 |
| | Anderson's gerbil (<i>Gerbillus andersoni</i>) | 3 | 0 | 0 | 0 | 0 |
| Wagner's gerbil (<i>Gerbillus dasyurus</i>) | 1 | 0 | 0 | 0 | 0 | |
| Canidae | Red fox (<i>Vulpes vulpes</i>) | 32 | 5 | 5/32 | 0 | 15.6 |
| | Golden jackal (<i>Canis aureus</i>) | 63 | 5 | 5/63 | 0 | 8 |
| Erinaceidae | Southern white-breasted hedgehog (<i>Erinaceus concolor</i>) | 3 | 0 | 0 | 0 | 0 |
| Total | | 402 | 28 | 25/402 | 9/57 | 7.2 |

DISCUSSION

This study of *B. persica* infection combines data on tick infection with findings on tick blood meals, identification of the animal species which infected ticks have fed on, and the wildlife animal hosts which harbor infection. The study has clearly identified an association between the presence of a blood meal and *B. persica* infection in *O. tholozani* ticks and has also shown that red fox and golden jackal blood meals are significantly associated with tick infection. These were supported by the relatively high rate of *B. persica* infection found in these two wild canine species surveyed along with other wildlife in this study. Only a very small number of unfed *O. tholozani* larvae were found to be infected with *B. persica*, while infection rates were significantly increased in the nymph and adult stages, in particular in those with a blood meal. These findings strengthen the idea that although transovarial transmission of *B. persica* exists, as previously reported (13), transmission associated with an animal reservoir probably plays an important role in the epidemiology of this infection, its maintenance, and its dispersal between remote tick foci. The existence of animal reservoirs for *B. persica* and the fact that its transmission is mainly transstadial and not transovarial were not known previously. Other TBRF *Borrelia* spp. are transmitted mostly transovarially in the tick, with some exceptions, such as *B. duttoni* transmitted by *Ornithodoros moubata* and *B. hermsii* transmitted by *Ornithodoros hermsi* (33–35). *Borrelia crucidurae* was shown to have a transovarial transmission rate of 33.3 to 53.3% in the soft tick *Ornithodoros erraticus* (36), and *Borrelia miyamotoi*, a TBRF transmitted by the hard tick *Ixodes scapularis*, was shown to have a transovarial transmission rate of 90.9% (37).

The collection of more than 51,000 ticks in multiple locations all over Israel has enabled this study to take an in-depth look at the natural history of *B. persica* infection. We documented a prevalence of *B. persica* in 2.6% in the soft ticks examined, ranging from 0.6 to 8.6% in different caves. A limited previous study reported a higher prevalence of *B. persica* in 8 of 94 (8.5%) *O. tholozani* ticks collected from 5 caves in Israel (30). Studies on argasid tick infection with other Old World relapsing fever *Borrelia* species have generally also reported higher infection rates, ranging between 4.6% to 20% (16, 38).

In contrast to some other relapsing fever borreliae, *B. persica* in Israel is prevalent in *O. tholozani* tick populations located in isolated niches of caves, ruins, or rock crevices, found distantly from each other with presumably no direct contact between these populations. This may explain the genetic variability observed between *O. tholozani* in this study as demonstrated by the phylogenetic analysis (Fig. S2). The detection of *O. tholozani* ticks in

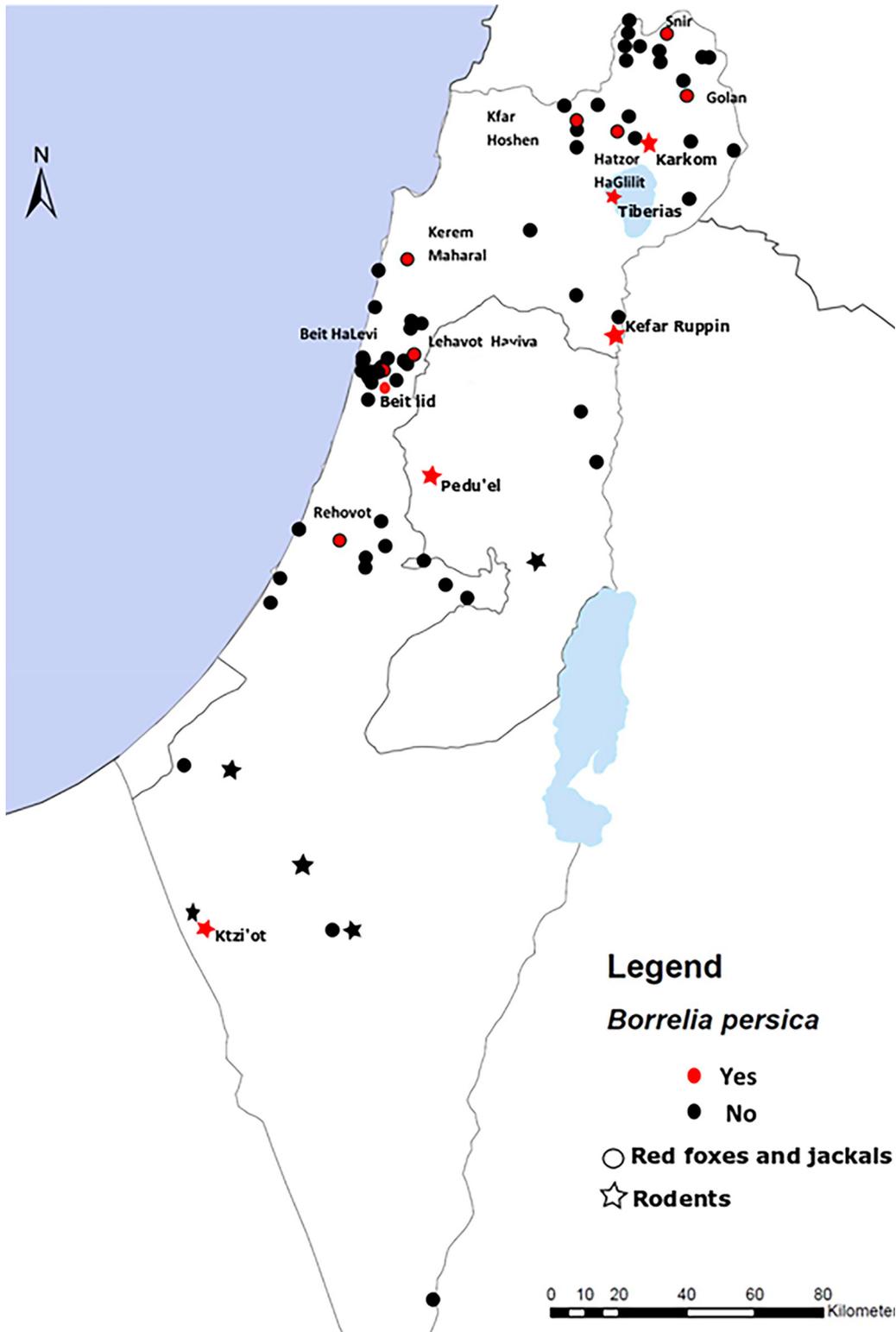


FIG 3 Locations where insectivores and wild canids were sampled, indicating the presence or absence of infection with *B. persica*. Locations where *B. persica* was detected are noted in red, and their names are included. Twelve collections sites corresponding to 12 wild canid samples were unknown, including an area where a positive sample was collected.

the surveyed locations indicated that they were present in 67% of the surveyed caves. Ticks were not found in the arid part of southern Israel represented by four caves (no. 21 to 24), probably due to incompatible climate conditions in agreement with previous studies on the prevalence of human TBRF in Israel and previous cave tick surveys (4, 9).

Regarding the association of certain animal blood meals in ticks and *B. persica* infection, since *O. tholozani* may take multiple blood meals from different animals in the same developmental stage, it may be difficult to confirm the association between a certain blood meal and a reservoir host. We therefore analyzed the association between blood meal and *B. persica* infection only in ticks in which a single host species blood meal was identified. Blood meal analysis revealed that although 74% of the blood meals found were from porcupines, only fox and jackal blood meals were strongly positively associated with infection with *B. persica*, and Cairo spiny mouse blood meals were also significantly associated with *B. persica* in ticks. The detection of a tick infected with "*Candidatus* *Borrelia fainii*," which is associated with bats and has been reported to be pathogenic to a human (32), was unexpected. Surprisingly, the infected tick harbored blood meal of a porcupine; however, several of the ticks examined from the same cave had bat blood meals (*Rousettus leschenaultia* and *R. aegyptiacus*). Thus, it is possible that the tick acquired this particular *Borrelia* sp. infection during a previous bat blood meal. The study of natural *B. persica* infection in wild animals revealed infection with *B. persica* in the social vole, Cairo spiny mouse, fat sand rat, red fox, and the golden jackal. Rodents serve as hosts for other TBRF *Borrelia* agents, including *B. caucasica*, *B. graingeri*, *B. hispanica*, *B. merionesi*, *B. coriaceae*, *B. hermsii*, *B. parkeri*, *B. turicatae*, *B. miyamotoi*, and *Borrelia crocidurae* (16, 39, 40). There are no records of natural rodent infection with *B. persica*; however, successful experimental infections of *B. persica* in laboratory guinea pigs and mice have been reported (18, 19). The highest rate of *B. persica* infection in rodents in our study was found in the social vole (22.4%), followed by the fat sand rat (8.3%) and Cairo spiny mouse (3%). Infection with *B. persica* was detected in 15.6% and 7.9% of the red foxes and golden jackals tested, respectively. Positive golden jackals originated from four locations, one from northern and three from central Israel, whereas positive red foxes originated from five sites, four from northern and one from central Israel. These findings further substantiate the significant association found between red fox and golden jackal tick blood meals and tick infection with *B. persica*, and they suggest that these wild canid species in Israel do not only harbor infection at relatively high rates but also contribute to infection of *O. tholozani* ticks and to the transmission from infected ticks to naive ticks.

While rodents tend to have a sedentary behavior and a relatively small habitat range for foraging and digging of burrows used for shelter, protection, and reproduction (41–43), golden jackals and red foxes show high geographical dispersal ranges in search of food in natural surroundings or waste deposited near human settlements (44). Thus, despite the fact that wild canids did not show the highest infection rates, it is highly likely that they have a main role in the transmission of *B. persica* to new and distant locations, infecting new ticks, which may infect other animals and humans who enter *O. tholozani* habitats.

An interaction between rodent and wild canid hosts of *B. persica* may allow the dissemination of *B. persica* locally and interregionally, with wild canids disseminating infection between distantly located *O. tholozani* populations and rodents maintaining permanent sources for transmission within a cave or a small area.

In conclusion, this study has mapped the widespread presence of *B. persica* infection in Israel, confirmed the status of *O. tholozani* as its tick vector, and found that transovarial transmission of this pathogen is limited and that infection of *O. tholozani* is related to feeding blood on animal hosts with foxes, jackals, and several rodent species as putative reservoirs for this infection. Control of *B. persica* TBRF may include enforcing inclusive measures to prevent entrance of animals to caves and other locations of *O. tholozani* abundance and warn visitors of the presence of dangerous ticks in order to minimize the risk of infection.

MATERIALS AND METHODS

Tick collection and study sites. Israel was divided into 12 study zones of 60 by 40 km representing the study areas (Fig. S4 in the supplemental material). Soft ticks were collected during 2014 and 2015 from a total of 24 caves (numbered 1 to 24), with two caves in each study area. Caves were selected as the sites of collection, since *O. tholozani* ticks are known to inhabit caves in Israel, and humans are typically infected when entering cave sites (4, 5). The caves selected for sampling included locations where human disease cases were reported to acquire TBRF ($n=8$), locations which were suspected of being

associated with human infection (where a clinical case of human TBRF was acquired after visiting the cave's area) ($n=4$), and locations from which there was no history of infection ($n=12$). The locations of the caves were obtained from the Israeli Cave Research Center at the Hebrew University (<https://www.malham.info/>), from the Israeli Health Ministry, and from the Amudanan website (<http://amudanan.co.il/>) (Fig. 1). CO₂ traps were used to trap ticks as described previously by Assus and Wilamowski (5). Tree collector traps connected to a cool box emitting CO₂ from dry ice were buried in the soil in each sampling site and left overnight. The next morning, all trapped ticks were collected, kept in vials with 70% alcohol, and brought to the laboratory for analysis. All the tick specimens were then identified morphologically as described by Filipova (45), counted, and sorted according to their life stage and gender if in the adult stage.

Animal samples. Samples from wildlife mammals, including canids, rodents, and hedgehogs, were collected from 2006 to 2018 by the Israeli Nature and Parks Authority as part of an epidemiological study on infection with *Leishmania tropica* and *Leishmania major* in wild animals and a rabies surveillance program as part of the Israeli national program of wildlife disease surveillance. All trapping locations were recorded with coordinates and plotted on a map. As trapping was made for reasons not related to the TBRF study, trapping locations were not necessarily in close proximity to tick collection sites; however, they covered all areas of Israel, including the northern, central, and southern parts. Small mammals were trapped using Tomahawk live box traps (Tomahawk Live Trap, Hazelhurst, WI, USA). Trapped animals were anesthetized by intramuscular injection of ketamine-HCl (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA, USA) at 10 mg/kg. Small rodents were euthanized using CO₂ chambers, and larger animals were euthanized by intravenous sodium pentobarbital injection and necropsied by the Israeli Nature and Parks Authority.

Blood was collected in EDTA tubes for DNA extraction and kept at -20°C for further analysis. Spleen tissue samples were collected during necropsy and kept frozen at -20°C until DNA extraction. Trapping of wild animals was performed by wardens of the Israel Nature and Park Authorities with an approved permit from this institution, and the study was conducted following the Hebrew University's guidelines for use of animals in research.

Molecular analysis. DNA from ticks was extracted using a commercial kit (DNeasy blood and tissue kit; Qiagen, Germany) following the manufacturer's protocol. PCR was performed on DNA extracted from 100 males, 100 females, and 100 nymphs from each cave. Larvae were analyzed both individually (50 per cave) and by pools (10 pools of five larvae each per cave). If a smaller number of ticks was available from a certain cave, then all individual ticks collected from each of the tick's life cycle stages from that cave were studied. DNA from animal blood (200 μl) was extracted using the Illustra blood genomicPrep mini spin kit (GE Healthcare, Buckinghamshire, UK), and DNA from splenic tissue was extracted by the guanidine thiocyanate technique, with some modifications as previously described (46).

PCR for *Borrelia* spp. All tick and animal DNA samples were screened for *Borrelia* infection by a real-time PCR targeting a 346-bp fragment of the *Borrelia* flagellin (*flaB*) gene using primers Fbpbu and Fbpccr (29) (Table 5). Ticks positive for *Borrelia* spp. were further tested for additional genotyping by PCR targeting a 750-bp segment of the same gene by conventional PCR with primers Bor1 and Bor2 (30) and by real-time PCR targeting a 280-bp fragment of the glycerophosphodiester phosphodiesterase gene (*glpQ*) using primers GlpQ-510f and GlpQ-770r (31) (Table 5). A conventional PCR mixture (25 μl) with DNA polymerase (Syntezza, Jerusalem, Israel) contained 4 μl target DNA, 0.4 μM of primers (Syntezza), and distilled water to reach the final volume. Real-time PCR assays targeting the *flaB* and *glpQ* genes were carried out in 20 μl Maxima Hot Start PCR master mix (Thermo Scientific, Loughborough, UK) with 0.25 μM each primer and 0.6 μl SYTO9 (Invitrogen, CA). The reactions were done at an annealing temperature of 52°C or 58°C for tick and animal samples, respectively, and 50 cycles followed by a melting phase. Plasmids (Topo TA cloning kit; Life Technologies, Grand Island, NY, USA) containing a *B. persica* *flaB* or *glpQ* gene insert, nontemplate control (NTC), and *Borrelia*-negative tick or animal DNA were employed as positive and negative controls, respectively.

PCR for host blood meal identification. The identification of tick blood meals was done as outlined in Fig. S5. General vertebrate primers (12S-16SF and 12S-16SR) targeting a 500-bp sequence that includes segments of the mitochondrial 12S and 16S rRNA genes were used for screening for the presence of blood meals in DNA extracted from tick samples (27) (Table 5). The DNA products of the positive ticks were sequenced, and the blood source was identified as described in the DNA sequencing and analysis section. Positive samples from this screening PCR, which showed an unknown host sequence or ambiguous DNA chromatography, were analyzed further for the possibility of a host whose sequences for the particular amplified DNA segments were missing from GenBank or for a sample containing a mixed blood meal. This was done using additional PCRs with species-specific primers targeting the mammal cytochrome *b*, mitochondrial 12S rRNA genes, or the 16S rRNA genes (Table 5). The products of these PCRs were also sequenced and identified. If the screening PCR showed a band which did not sequence well due to insufficient amplified DNA, an additional primer set (16S24F and 16S366R) targeting a 366-bp internal segment of the 16S rRNA gene designed for this study based on alignments of the segment previously described (500-bp segment, including the mitochondrial 12S and 16S rRNA gene) was used for a nested PCR (Table 5). Conventional PCRs for tick blood meal identification were performed in a mixture (25 μl) with DNA polymerase (Syntezza) containing 3 μl of target DNA and 0.4 μM concentrations of primers (Syntezza). For nested PCR, 1 μl of DNA product was used as the template. The protocol used for the PCR screening of blood meals targeting mitochondrial 12S and 16S rRNA genes was performed as described by Valinsky et al. (27), with a modified annealing temperature of 60°C . The cycling parameters for the nested PCR were 94°C for 2 min followed by 35 cycles of 40 s at 94°C , 40 s at

TABLE 5 PCR primers used for tick identification, detection of *Borrelia* spp., and identification of blood meal sources

| Primer | Target gene | Target species | 5'-3' primer sequence | Amplicon length (bp) | Reference no. or source |
|--|---------------------------------------|---------------------|-------------------------------|----------------------|-------------------------|
| Primers for tick identification | | | | | |
| 16S+1 | 16S rRNA | Ticks | CTGCTCAATGATTTTTTAAATTGCTGTGG | 480 | 47 |
| 16S-1 | 16S rRNA | Ticks | CCGGTCTGAACCTCAGATCAAGT | 480 | 47 |
| Primers for <i>Borrelia</i> sp. identification | | | | | |
| Bfpbu | <i>flaB</i> | <i>Borrelia</i> | GCTGAAGAGCTTGGAAATGCAACC | 346 | 29 |
| Bfpcr | <i>flaB</i> | <i>Borrelia</i> | TGATCAGTTATCATTCTAATAGCA | 346 | 29 |
| BOR1 | <i>flaB</i> | <i>Borrelia</i> | TAATACGTCAGCCATAAATGC | 750 | 30 |
| BOR2 | <i>flaB</i> | <i>Borrelia</i> | GCTCTTTGATCAGTTATCATTC | 750 | 30 |
| GlpQ-510f | <i>glpQ</i> | <i>Borrelia</i> | AAAACCCCTTTGGCATAAACAAACA | 280 | 31 |
| GlpQ-770r | <i>glpQ</i> | <i>Borrelia</i> | CCAGGGTCCAATTCCTCAG | 280 | 31 |
| Primers for identification of blood meal | | | | | |
| 12S-16SF | 12S and 16S rRNA | All vertebrates | ACACCGCCCGTCACCCCTCC | 500 | 27 |
| 12S-16SR | 12S and 16S rRNA | All vertebrates | AACCAGCTATCACCAGGCTCG | 500 | 27 |
| 16S24F | 16S rRNA, inner primer for nested PCR | All vertebrates | AAGTCGTAACATGGTAAGCA | 366 | This study |
| 16S366R | 16S rRNA, inner primer for nested PCR | All vertebrates | GGTAGCTCGTCTGGTTCCGG | 366 | This study |
| Por100f | 16S rRNA | Porcupine | CAGAAGACTTCAGGTAATACTGGC | 311 | This study |
| Por391r | 16S rRNA | Porcupine | GGGTGGCTCAATTTAGCTGT | 311 | This study |
| FOX131f | 16S rRNA | Fox | ACGAAAGCTAGCCCAATCGAC | 303 | This study |
| FOX410r | 16S rRNA | Fox | CACTATTTGGCCACATAGACGAGT | 303 | This study |
| BADG45f | 16S rRNA | Badger | ACATCATGACCCTTTGAACCA | 302 | This study |
| BADG345r | 16S rRNA | Badger | TGAGTTTATCCCTGTGGATTGT | 302 | This study |
| Jack134f | 16S rRNA | Jackal | GCTAGCCCAACTAACCCCAA | 287 | This study |
| Jack401r | 16S rRNA | Jackal | TTTTGCTACATAGATGAGTTGATCC | 287 | This study |
| Hyrax102f | 16S rRNA | Hyrax | AACCCATTGACCACCTTGAACCT | 305 | This study |
| Hyrax407r | 16S rRNA | Hyrax | GGGTGGTCTGTAACTGCTC | 305 | This study |
| Cat95f | 16S rRNA | Cat | TCATATTAACCTGACCCTTTGAGC | 318 | This study |
| Cat412r | 16S rRNA | Cat | CAACATAGACGAGTTCATCCTGTAA | 318 | This study |
| goat 162F | 16S rRNA | Goat | TTACCAAAACAGTCTAAACAA | 238 | This study |
| Goat380r | 16S rRNA | Goat | AACTGCCATGAGTAGCTCG | 238 | This study |
| Unrev1025 | Cytochrome <i>b</i> | Mammal | GGTTGCTCTCCAATTCATGTTA | 334/680 ^c | 26 |
| Human741F ^a | Cytochrome <i>b</i> | Human | GGCTACTTCTCTTCATTCTCTCT | 334 | 26 |
| Dog368F ^a | Cytochrome <i>b</i> | Dog | GGAATTGTACTATTATTCGCAACCAT | 680 | 26 |
| 12S-13R | 12S rRNA | All vertebrates | AGGAGGGTGACGGGCGGT | 326/369 ^d | 24 |
| RodentF ^b | 12S rRNA | Muridae, Cricetidae | GGCGGTACTTTATATCCAT | 326 | 24 |
| BirdF ^b | 12S rRNA | Birds | TACGAGCACAAACGCTTAA | 369 | 24 |

^aForward primers used with UNREV1025.

^bForward primers used with 12S-13R.

^cReverse primer UNREV amplifies a 334-bp amplicon with the forward primer Human741F when amplifying a human blood meal and a 680-bp amplicon with the forward primer Dog368F when amplifying a dog blood meal.

^dReverse primer 12S-13R amplifies a 326-bp amplicon with the forward primer RodentF when amplifying a rodent blood meal and a 369-bp amplicon with the forward primer BirdF when amplifying a bird blood meal.

62°C, and 40 s at 72°C and 4 min at 72°C. PCR using species-specific primers was performed as for the detection of mitochondrial genes in a blood meal (27). NTCs as negative and mammal positive controls were included in each PCR.

The validation of efficacy of detection of different blood meals was performed by testing the primers against DNA of the following mammals from which DNA was present in our laboratory: dog (*Canis lupus familiaris*), cow (*Bos taurus*), cat (*Felis catus*), camel (*Camelus dromedaries*), rock hyrax (*Procapra capensis*), red fox (*Vulpes vulpes*), golden jackal (*Canis aureus*), porcupine (*Hystrix indica*), gerbil (*Meriones unguiculatus*), Cairo spiny mouse (*Acomys cahirinus*), sand rat (*Psammomys obesus*), human, and the house mouse (*Mus musculus*), as well as DNA from engorged field-collected soft ticks. All of the primers used successfully amplified the animal species tested.

PCR for tick characterization. All *Borrelia*-positive tick DNA samples and 10 *Borrelia*-negative samples from each cave were analyzed by PCR targeting a 460-bp segment of the tick mitochondrial 16S rRNA gene using primers 16S+1 and 16S-1 (Table 5). The PCR protocol for tick 16S rRNA gene detection was

performed according to a previous publication with a modified annealing temperature set at 60°C (47). The PCR mixture (25 µl) contained 3 µl of target DNA and 0.4 µM of primers (Syntezza). NTC and *O. tholozani* tick DNA used as negative and positive controls, respectively, were included in each PCR.

New primers for tick blood meal identification were designed using the Primer-BLAST website (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The primers were tested with DNA of mammals from which DNA was present in our laboratory as detailed earlier. The other primers used were from previously published articles (Table 5). Nevertheless, all primers were standardized with the DNA polymerase and thermocycler used in this study, and all positive samples were sequenced to confirm the identity of the blood meal source.

DNA sequencing and analysis. All samples positive by PCR in this study underwent DNA sequencing. Phylograms were constructed using the maximum-likelihood algorithm in the MEGA software, version X (48). Amplified conventional PCR products were detected by electrophoresis on 2% agarose gel stained with ethidium bromide. PCR amplicons of conventional PCR and positive real-time products were purified using a PCR purification kit (ExoSAP; New England Biolabs, Inc., Ipswich, MA) and subsequently sequenced using the Sanger technique at the Center for Genomic Technologies (The Hebrew University, Jerusalem, Israel). Species-level identification was obtained when the sequences were the first match by BLAST and showed percentage identity higher or equal to 98%. All DNA sequences were aligned using MUSCLE in the MEGA software version X. The sequences were further compared with sequences from GenBank using the BLAST algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic analysis was performed by MEGA software, version X. Phylograms were constructed using the maximum-likelihood, Tamura 3-parameter model for the *Borrelia flaB*, Tamura-Nei model for *glpQ* sequences, and the Hasegawa-Kishino-Yano model for tick 16S rRNA sequences. Phylogram robustness was determined by bootstrap (BS) percentages with 1,000 replicates.

Data source and statistical analysis. Chi-square, Fisher's test, and Student's *t* test were used for univariable models for nominal and ordinal variables, respectively. These tests were used to find an association between tick *B. persica* infection, tick life stage, presence of blood meal, and blood meal source. Statistical analysis was performed using WinPepi (version 11.65) and SPSS software (version 20; IBM, New York, NY, USA).

Data availability. DNA sequences generated in this study were deposited in GenBank under the following accession numbers: [KX258784](https://www.ncbi.nlm.nih.gov/nuclot/KX258784) to [KX258797](https://www.ncbi.nlm.nih.gov/nuclot/KX258797) for *B. persica flaB* partial sequences and [MK636871](https://www.ncbi.nlm.nih.gov/nuclot/MK636871) for an uncultured *Borrelia* sp. *flaB* partial sequence from ticks, [MK138356](https://www.ncbi.nlm.nih.gov/nuclot/MK138356) and [MK138357](https://www.ncbi.nlm.nih.gov/nuclot/MK138357) for *flaB* partial sequences from wildlife, [MH923346](https://www.ncbi.nlm.nih.gov/nuclot/MH923346) to [MH923348](https://www.ncbi.nlm.nih.gov/nuclot/MH923348) for *B. persica glpQ* partial sequences from ticks, and [MF615990](https://www.ncbi.nlm.nih.gov/nuclot/MF615990) to [MF615997](https://www.ncbi.nlm.nih.gov/nuclot/MF615997) for *O. tholozani* tick 16S rRNA gene partial sequences.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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