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Transmission Dynamics of *Bartonella* sp. Strain OE 1-1 in Sundevall's Jirds (*Meriones crassus*)

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A high prevalence of *Bartonella* infection is found in many natural systems; however, the transmission dynamics leading to observations of these infections is not fully understood. The capability of *Xenopsylla ramesis* fleas to serve as competent vectors of *Bartonella* sp. OE 1-1 (a strain closely related to the zoonotic *Bartonella elizabethae*) to *Meriones crassus* jirds was investigated. Naïve *X. ramesis* fleas were placed for 72 h on naïve jirds or jirds that were either experimentally or naturally infected with *Bartonella* sp. strain OE 1-1, after which they were placed on naïve jirds. Postfeeding, 69 to 100% of the fleas collected from each *Bartonella*-positive jird contained *Bartonella* DNA, and all naïve jirds became positive for *Bartonella* sp. OE 1-1 after infestation with the infected fleas. In addition, maternal transmission of *Bartonella* sp. OE 1-1 in jirds was tested by mating 5 *Bartonella*-positive and 5 naïve female jirds with 10 naïve male jirds in the absence of fleas. Fifteen offspring were delivered by each group. Cultures of blood drawn from all offspring on days 35 and 47 postdelivery were found to be negative for *Bartonella*. A single spleen sample from the offspring of a *Bartonella*-positive mother was found molecularly positive for *Bartonella* sp. OE 1-1. This study demonstrates that *X. ramesis* fleas are competent vectors of *Bartonella* sp. OE 1-1 to *M. crassus* jirds and indicates that maternal transmission is probably not the major transmission route from female jirds to their offspring. We suggest that the dynamics of *Bartonella* sp. OE 1-1 in the *M. crassus* jird population in nature is mostly dependent on its vectors.

Bartonella species are fastidious Gram-negative bacteria capable of causing persistent bacteremia in humans and other mammals (1). They are transmitted from one host to another by blood-sucking arthropods (2). In recent years, increasing numbers of *Bartonella* species and genotypes have been detected in a wide number of hosts and vectors, mainly rodents and their fleas. Only a few were confirmed experimentally as competent vectors, and sound information on the transmission routes of *Bartonella* spp. is lacking (3).

Several *Bartonella* spp. of rodent origin have been associated with human illness. These include *Bartonella elizabethae*, *Bartonella grahamii*, *Bartonella vinsonii* subsp. *arupensis*, *Bartonella washoensis*, *Bartonella tamiae* (4), and *Bartonella tribocorum* (5). Investigation of the transmission routes by competence studies and elucidation of the dispersal dynamics of *Bartonella* among animals and their vectors are crucial for understanding how these organisms are being maintained in nature and how they cause illness. A blood-sucking arthropod has to be capable of acquiring, maintaining, and transmitting a specific microbial agent to a specific host in order to be considered a competent vector (6). Its ability to transmit an infectious agent to a specific host depends on two factors: it must allow the pathogen to survive in its body and be transmitted as a viable organism to a new host. Proven and putative vectors of *Bartonella* species were reviewed recently (7).

Bartonella dynamics in nature is substantially influenced by the variety and number of competent vectors in a specific region. In previous studies, *Bartonella* sp. strain OE 1-1, a strain closely related to the zoonotic *Bartonella elizabethae*, was detected in blood collected from *Meriones crassus* jirds and *Gerbillus nanus* gerbils from Israel (8) and *Gerbillus pyramidum* gerbils from Egypt (9). In another study, we detected a high prevalence of this *Bartonella* strain in several flea species collected from several rodent species (10), suggesting that it is a common *Bartonella* strain in rodents and fleas in this region. In the latter study, 6 distinct *Bartonella* genotypes were identified in *X. ramesis* fleas, necessitating further investigation of the capability of *X. ramesis* to serve as a competent vector for these genotypes (10).

Several mammalian hosts have been shown to contribute to the bacterium's dispersal by maternal (vertical) transmission. A previous study reported that *Bartonella* spp. designated as groups A and D are transmitted vertically from wild-captured female cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) to their offspring (11). Another study reported similar findings in BALB/c mice infected with *Bartonella birtlesii* (12). Conversely, no maternal transmission of *Bartonella grahamii* and *Bartonella taylorii* was reported in female bank voles (3). The aim of the current study was to expand our knowledge on the dispersal dynamics of *Bartonella* in the wild rodent population by investigating whether *X. ramesis* is a competent vector of *Bartonella* sp. OE 1-1 to *M. crassus* jirds and to elucidate whether this *Bartonella* sp. can be transmitted maternally from the mother jird to its offspring.

MATERIALS AND METHODS

***Bartonella* sp. OE 1-1.** The *Bartonella* sp. OE 1-1 isolate used in this study was obtained from a naturally infected Sundevall's jird from Israel (8); the

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TABLE 1 Infection of fleas and jirds with *Bartonella*

No. of fleas applied		Jird to which fleas were applied (day 15)[a,b]	No. of fleas collected on day 18		Jird to which fleas were transferred (day 19)[c]	No. of female fleas collected (day 22)	No. (%) of *Bartonella*-positive female fleas	No. of male fleas collected (day 22)	No. (%) of *Bartonella*-positive male fleas
Female	Male		Female	Male					
40	20	MN1	37	13	MN16	29	0 (0)	11	0 (0)
40	20	MN2	36	20	MN18	33	0 (0)	18	0 (0)
40	20	MN3	40	19	MN17	38	0 (0)	17	0 (0)
40	20	MN4	33	17	—[e]	28[d]	0 (0)[d]	13[d]	0 (0)[d]
40	20	MN5	36	20	—	30[d]	0 (0)[d]	18[d]	0 (0)[d]
40	20	MN6	39	12	—	33[d]	0 (0)[d]	9[d]	0 (0)[d]
40	20	MP7	30	12	MN3	24	21 (88)	8	7 (88)
40	20	MP8	33	15	MN1	28	21 (75)	13	11 (85)
40	20	MB9	37	20	MN4	37	36 (97)	19	16 (84)
40	20	MB10	37	19	MN6	35	29 (83)	17	16 (94)
40	20	MB11	36	20	MN5	28	28 (100)	17	13 (76)
40	20	MB12	29	20	MN2	23	20 (87)	14	12 (86)
40	20	MB13	34	19	MN15	33	28 (85)	16	11 (69)
40	20	MB14	29	13	—	22[d]	19 (86)[d]	12[d]	9 (75)[d]

[a] MN1 to MN6 represent *Bartonella*-negative jirds, MP7 and MP8 represent jirds naturally infected with *Bartonella*, and MB9 to MB14 represent jirds experimentally infected with *Bartonella*.
[b] On day 15, fleas were placed on jirds and collected after 72 h.
[c] On day 19, fleas that had been previously placed on naïve or *Bartonella*-positive jirds were transferred to naïve jirds.
[d] Fleas that were subjected to *Bartonella* PCR without being transferred to naïve jirds for a second infestation period.
[e] —, fleas were not transferred to new jirds but were subjected to PCR.

isolate was cultured on chocolate agar plates in a 5% CO_2 incubator (model no. 3111; Thermo Forma, Ohio) at 37°C. Bacterial colonies used were passaged twice before inoculation (first-passage colonies were harvested after 20 days and then reinoculated and harvested again 14 days later). The colonies were added into a solution containing 80% Luria-Bertani medium (Difco Microbiology, Kansas) and 20% glycerol and were kept frozen at −80°C until thawed and used for inoculation. The bacterial concentration of each inoculation dose was determined by a series of 10-fold dilutions. The isolate used was confirmed to be identical to *Bartonella* sp. OE 1-1 by PCR and high-resolution melt (HRM) real-time quantitative PCR. The *Bartonella* sp. OE 1-1 citrate synthase (*gltA*) gene sequence is in GenBank (under accession number HM771297).

Inoculation of jirds with *Bartonella* sp. OE 1-1. Ten male and 18 naïve female *M. crassus* jirds (Sundevall's jirds), captured in the Negev Desert in Israel, were used in this study (see Table S1 in the supplemental material). They were screened for *Bartonella* infection by 2 consecutive blood cultures, 14 days apart, and by PCR targeting the *gltA* and the RNA polymerase B (*rpoB*) genes as described previously (8), and they were confirmed to be free of *Bartonella* spp. Six of the female jirds (MB9 to MB14) were subcutaneously inoculated with 10^7 CFU of *Bartonella* sp. OE 1-1 diluted in 1.0 ml phosphate-buffered saline (PBS). Six other naïve jirds (MN1 to MN6) were used as negative controls and injected subcutaneously with 1.0 ml PBS. Another 6 naïve female jirds (MN15 to MN20) were used for succeeding stages of the study. Two additional female jirds (MP7 and MP8; captured in the Negev desert) naturally infected with *Bartonella* sp. OE 1-1, as confirmed by blood cultures and molecular screening of the colonies, were also included in the study. This study was carried out in strict accordance with the recommendations in the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (13) and approved by The Israel Nature and National Parks Protection Authority (approval number 2011/38279) and Hebrew University's Animal Care and Use Committee (approval number MD-10-12720-3).

Fleas. *Bartonella*-free *X. ramesis* fleas, maintained as described previously (14, 15), were used in this study. Briefly, 3 *Bartonella*-free Sundevall's jirds were placed in individual cages containing a steel nest box with a screen floor and a pan with a mixture of autoclaved sand and dried bovine blood that was examined and verified to be *Bartonella*-free using PCR, as described below. The jirds were infested with 10 to 15 newly emerged fleas. Every 2 weeks, all substrate and bedding material was collected from the cage and transferred into an incubator (model FOC225E; Velp Scientifica srl, Milano, Italy), where flea emergence and development took place at 25°C and 75% relative humidity.

Acquisition of *Bartonella* by fleas and transmission to naïve jirds. Forty newly emerged female and 20 newly emerged male laboratory-reared naïve *X. ramesis* fleas were placed on each of the 6 experimentally infected female jirds (MB9 to MB14) when they were confirmed to be bacteremic (day 15 postinoculation), as well as on the 2 naturally infected *M. crassus* jirds (MP7 and MP8) and the 6 naïve female jirds (MN1 to MN6) (Table 1). After 72 h, the fleas were collected manually and separately from each jird and its cage substrate and transferred into a disinfected Erlenmeyer flask for overnight incubation (10 to 12 h at 25°C and 75% relative humidity). The following morning, each flea pool collected from 5 experimentally infected jirds (MB9 to MB13) and from the 2 naturally infected jirds (MP7 and MP8) was placed on a single naïve jird (MN1 to MN6 and MN15) for another 72 h. The flea pool from the sixth experimentally infected jird (MB14) was screened for *Bartonella* using PCR as described below. Flea pools collected from 3 *Bartonella*-naïve jirds (MN1 to MN3) were placed separately on another 3 *Bartonella*-naïve jirds (MN16 to MN18) for 72 h. Fleas collected from 3 other *Bartonella*-naïve jirds (MN4 to MN6) were screened for *Bartonella* using PCR (Table 1).

Maternal transmission of *Bartonella* in female jirds. Following the vector competence experiment, 5 culture-confirmed *Bartonella*-positive female jirds (group 1: MB9 to MB13) and 5 *Bartonella*-negative female jirds (group 2: MN16 to MN20) were mated with 10 *Bartonella*-negative male jirds. Each jird couple was kept in a separate cage and free of fleas. All jirds were thoroughly examined and confirmed to be free of fleas. In addition, one drop of fipronil (Frontline; Merial, Georgia) was applied to the skin of each jird between the scapulae to prevent flea infestation. After copulation, the males were removed from the cages, and the pregnant females were kept separately until delivery took place. Postdelivery, the females remained with their offspring for 4 weeks. Thereafter, the offspring were separated into individual cages and maintained for an additional 2 months. The jirds in this study were housed under laboratory conditions as described previously (14, 16). In brief, each jird was main-

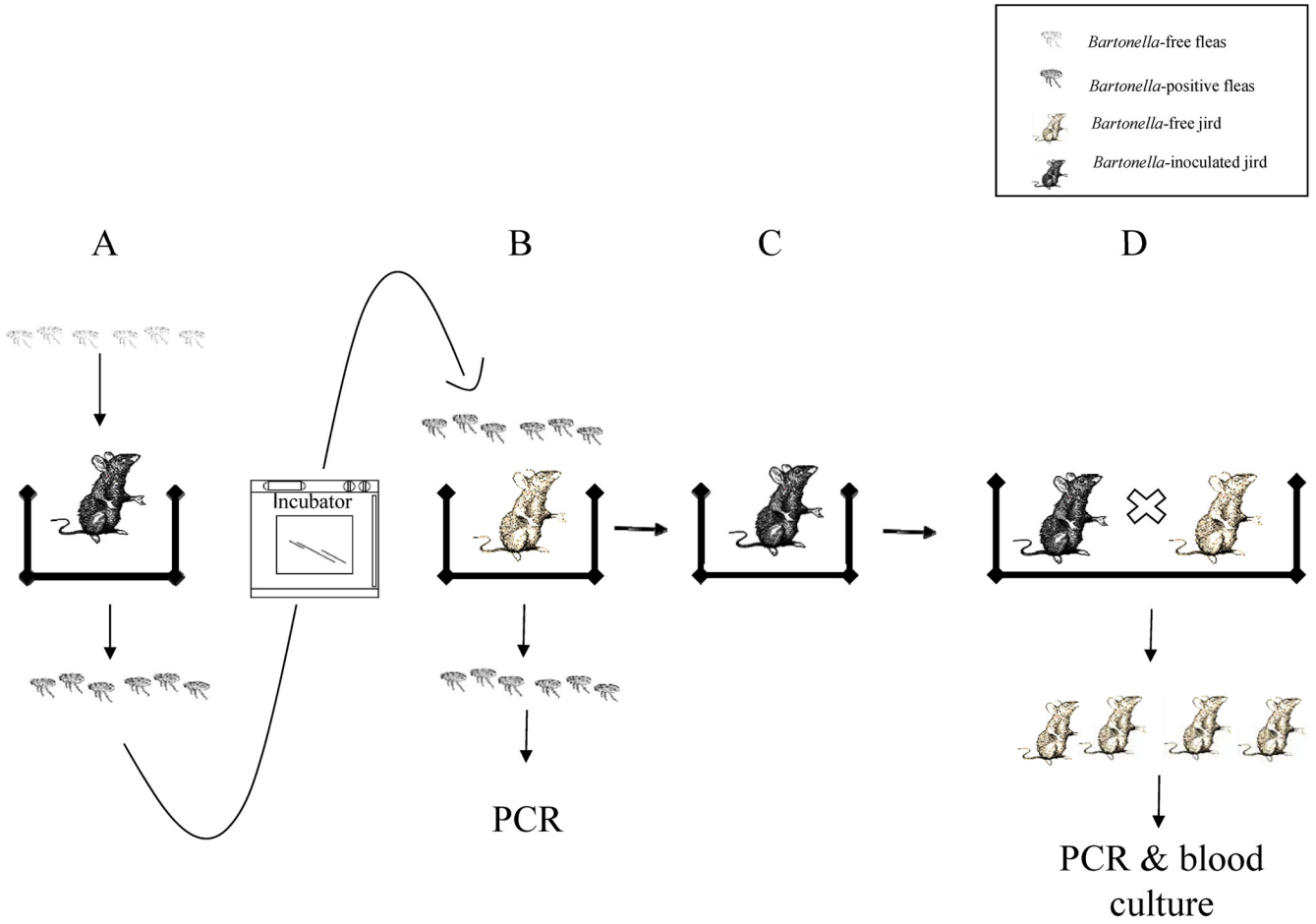

FIG 1 Schematic description of the basic experimental design (shown for one jird). (A) Forty female and 20 male naïve fleas were placed on each of 6 jirds experimentally inoculated with *Bartonella* and on 2 naturally infected jirds on day 15 for 72 h. (B) Fleas collected from *Bartonella*-inoculated jirds were placed on *Bartonella*-free jirds for another 72 h. (C) *Bartonella*-free jirds became bacteremic after infestation with the fleas. (D) *Bartonella*-inoculated female jirds were mated with *Bartonella*-free male jirds and produced offspring.

tained separately in a plastic cage (60 by 50 by 40 cm) and offered millet seed and fresh alfalfa (*Medicago* sp.) *ad libitum*. They were inspected daily for observable clinical abnormalities. Three months after delivery, all offspring were euthanized. Tissue samples from the lung, liver, kidney, spleen, and brain from each offspring were collected during necropsy (Fig. 1).

***Bartonella* isolation and DNA extraction.** During the course of the 245-day study, blood was periodically collected (into EDTA tubes) from the orbital sinus of each jird (15 blood collections per jird) under isoflurane general anesthesia (see Table S1 in the supplemental material). On days 35 and 47 postdelivery, blood was collected from all jird offspring of the 2 groups. Two hundred microliters of blood was cultured in an incubator on a chocolate agar plate for up to 11 weeks in 5% CO_2 at 37°C. *Bartonella* colonies were diagnosed morphologically as small creamy-white colonies. For each positive plate, the number of colonies per plate was counted. The numbers of colonies counted were rounded to the nearest multiple of 10, and when more than 1,000 colonies were counted, they were reported as 1,000 (see Table S1).

DNA was extracted from *Bartonella* colonies using a DNA extraction kit (Illustra tissue minispin kit; GE Healthcare, Little Chalfont, United Kingdom) according to the manufacturer's instructions and confirmed for *Bartonella* sp. OE 1-1 using PCR and sequencing as described below.

Fleas were collected 3 days after infestation of the naïve jirds and were kept in 70% ethyl alcohol until further analyzed. Each single flea was sliced into minute pieces by a separate new sterile scalpel blade. DNA was extracted separately from each single flea using the DNA extraction kit described above.

Following necropsy of the jird offspring, DNA was extracted from 50 mg of each tested tissue by the DNA extraction kit described above and screened molecularly as described below.

PCR analysis and sequencing. Molecular screening for *Bartonella* in the fleas and cultured colonies was carried out using conventional PCR with Bhcs.781p and Bhcs.1137n primers targeting the 313-bp fragment of the *gltA* gene (17, 18). The PCR procedure was performed in a 50-μl reaction volume containing 2 μl of DNA, 20 pmol of each primer, 21 μl of double-distilled water (DDW), and 25 μl of PCR-Ready high specificity (Syntezza, Jerusalem, Israel). For tissue samples, HRM real-time PCR analysis was performed using primers 600f and 800r targeting a 194-bp fragment of the *rpoB* gene (19). DNA extracted from a cultured *Bartonella* Tel Aviv Rr. strain (GenBank accession number FJ577651) was used as a positive control, and two samples containing all the ingredients of the reaction mixture except DNA were used as negative controls for all PCR procedures. Randomly chosen *Bartonella*-positive samples of blood cultures and fleas (2 to 3 fleas, minimum) from each flea group were selected for further sequence analysis. Amplicons were purified using the ExoSAP-IT PCR purification kit (Affymetrix, Santa Clara, CA). DNA sequencing was carried out utilizing the BigDye Terminator cycle sequencing chemistry (Applied Biosystems, Foster City, CA), the ABI 3700 DNA

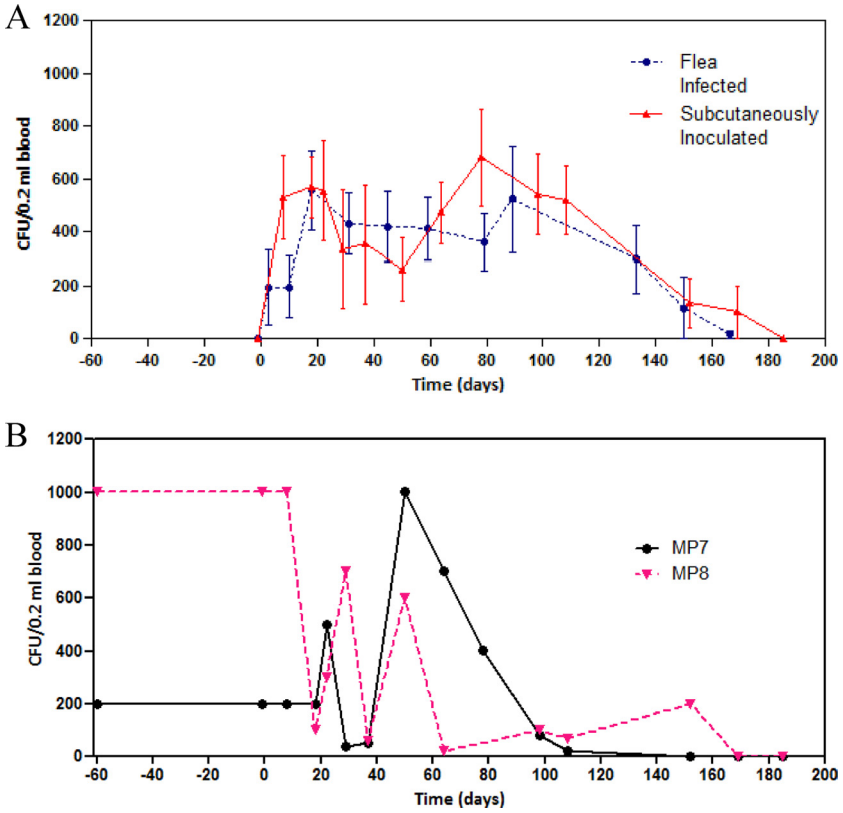

FIG 2 Mean bacteremia levels of flea-infected, subcutaneously inoculated, and naturally infected jirds. (A) Mean bacteremia levels (in numbers of CFU) in jirds throughout the study (245 days). Shown is a normalized graph comparing the mean bacteremia levels between jirds infested with *Bartonella*-infected fleas ($n = 7$) and jirds subcutaneously inoculated with *Bartonella* sp. OE 1-1 ($n = 6$). Vertical lines represent standard errors. (B) Bacteremia in jirds naturally infected with *Bartonella* sp. OE 1-1 ($n = 2$).

analyzer (Applied Biosystems), and the accompanying data collection and sequence analysis software. Further analysis was performed using the Sequencher software, version 4.8 for Mac (Gene Codes Corporation, Ann Arbor, MI). The obtained sequences were analyzed initially by BLAST through the NCBI's Mega-BLAST algorithm and further aligned with other *Bartonella* sequences using ClustalW 1.83 (MegAlign; DNAStar Lasergene software).

RESULTS

Inoculation of jirds with *Bartonella* sp. OE 1-1. All 6 inoculated jirds (MB9 to MB14) became infected with *Bartonella* sp. OE 1-1 as confirmed by blood cultures and molecular screening of the colonies. Bacteremia lasted for 100 to 161 days. The mean bacteremia levels of the 6 jirds increased rapidly through day 18. A slight decrease occurred thereafter, followed by another increase peaking on day 78. Then, the mean bacteremia levels started decreasing until day 185, when all jirds became *Bartonella* negative (Fig. 2A). In this jirds group, the bacteremia levels ranged from 30 to more than 1,000 colonies per plate (see Table S1 in the supplemental material). The 6 *Bartonella*-naïve jirds (MN1 to MN6) that were injected with PBS and served as controls for the first stage of the study remained negative at this stage.

Acquisition of *Bartonella* by fleas. During flea collection at the different stages of the study, not all applied fleas could be collected (Table 1), as some were probably eaten by the hosting jird. Sixty-nine to 100% of the individual fleas, collected from each *Bartonella*-positive flea-infected jird (MP7 and MP8 and MB9 to MB14) (Table 1), contained *Bartonella* sp. OE 1-1 DNA. The infection rate of female fleas (88%) was slightly higher than that of the male fleas (82%) but did not differ significantly (Mann Whitney test, $P = 0.2265$) (Table 1). The fleas which were collected from one *Bartonella*-positive jird (MB14) and were not placed on a naïve jird showed the same trend, with mean infection rates of 86% for female fleas and 75% for male fleas. All fleas that infested *Bartonella*-negative jirds remained negative during the entire experiment. All obtained sequences of amplified *Bartonella* DNA from blood cultures and fleas were identical and identified as *Bartonella* sp. OE 1-1.

Transmission of *Bartonella* to naïve jirds. All 5 naïve jirds (MN2, MN4 to MN6, and MN15) that were infested for 72 h with the fleas that had been previously placed on the *Bartonella*-inoculated jirds (MB9 to MB14) became bacteremic (see Table S1 in the supplemental material). The 2 naïve jirds (MN1 and MN3) that were infested with the fleas that had been previously placed on the 2 jirds that were naturally infected with *Bartonella* (MP7 and MP8) became bacteremic as well. All 3 naïve jirds (MN16 to MN18) that were infested with fleas that had been previously placed on naïve jirds (MN1 to MN3) remained negative.

The kinetics of bacteremia of the jirds that were infested with *Bartonella*-infected fleas was similar to that of the jirds that were experimentally inoculated by subcutaneous injection of *Bartonella* (Fig. 2A). The flea-infected jirds showed slower increases in bacteremia levels from day 19 until day 37 (18 days postapplica-

tion of the fleas). Then, the levels of bacteremia were relatively stable until day 108 (89 days postapplication of fleas), after which they started to decline until day 169 (150 days postapplication of fleas), when most jirds (except MN2) became *Bartonella* negative (see Table S1 in the supplemental material). In this group, the bacteremia levels ranged from 20 to more than 1,000 colonies per plate (see Table S1).

The two naturally infected jirds (MP7 and MP8) were bacteremic for at least 212 and 168 days, respectively (see Table S1). Distinctly different bacteremia patterns were observed in these two jirds (Fig. 2B). Their bacteremia levels ranged from 20 to more than 1,000 colonies per plate (see Table S1).

Transmission of *Bartonella* from female jirds to their offspring. Following pregnancy, the *Bartonella*-positive female jirds delivered 15 live offspring (MB9 delivered 4 males; MB10, 5 males and 1 female; and MB12, 3 males and 2 females). The *Bartonella*-negative female jirds delivered 11 live offspring (MN17, 2 males and 2 females; MN18, 1 female; and MN20, 4 males and 2 females). In this group, the morning following the delivery, an additional 4 dead pups were found in one female jird's (MN19) cage (2 males and 2 partially bitten offspring that were severely damaged, so their sex could not be determined). The male-to-female ratio was higher in the *Bartonella*-positive female jird group (12:3) than in the *Bartonella*-negative female jird group (8:5); however, the difference was not found to be significant (Fisher's exact test, $P = 0.218$). When the data were reanalyzed including the 2 bitten offspring with unknown genders as possibly 2 males, 2 females, or 1 female and 1 male, the difference was still found to be nonsignificant (Fisher's exact test, $P = 0.68$, 0.245, and 0.427, respectively). Cultures from blood drawn on days 35 and 47 postpartum from all of the offspring were negative for *Bartonella*. All offspring tissues that were molecularly screened for *Bartonella* DNA were found to be negative except for one spleen sample from a male offspring of a *Bartonella*-positive jird (MB10).

DISCUSSION

In this study, the transmission dynamics of *Bartonella* sp. OE 1-1 in Sundevall's Jirds (*M. crassus*) was investigated. *Xenopsylla ramesis* fleas were found to be competent vectors of *Bartonella* sp. OE 1-1 to *M. crassus* jirds. A period of 72 h was sufficient to acquire *Bartonella* from positive bacteremic jirds, and the same period was sufficient for transmission of the bacteria to naïve jirds. The high percentage (69 to 100%) of fleas that acquired *Bartonella* sp. OE 1-1 suggests the great efficiency of this vector in acquiring this *Bartonella* species. Male and female fleas did not differ in their capability to acquire *Bartonella*, as we noted in our previous study (10), indicating that both flea sexes are capable of acquiring *Bartonella* efficiently. Our results are similar to those of a previous study where *B. grahamii* and *B. taylorii* were detected in 21 out of 28 bank voles after *Bartonella*-positive fleas were added to their arena (3). In the same study, all 10 flea pools examined were *Bartonella* positive, and 7 of 10 randomly collected individual fleas were also positive.

In our study, all naïve jirds became infected following the application of *Bartonella*-infected fleas. The higher rodent infection rate in our study was probably due to the application of more fleas per jird and the separation of each jird and its fleas in a single cage rather than using one common arena. To the best of our knowledge, no information regarding the minimal number of fleas required for the transmission of *Bartonella* is present in the biological-medical literature. In our study, the number of *Bartonella*-positive fleas applied to naïve jirds varied from 1 jird to another. It can be noted that out of 42 fleas placed on jird MN3, 32 were collected after 72 h, and 28 of them were found to be *Bartonella* positive, indicating that 28 to 38 fleas (the latter number includes the 10 fleas that were probably eaten) were sufficient to transmit this *Bartonella* species (Table 1). It should be noted that the number of fleas on a rodent host in nature varies, depending on the rodent species, the flea species, the habitat type, and the season (20). Fleas in nature are known to spend considerably more time on a host than is required to obtain a blood meal, but they also spend a significant amount of time off-host in their burrows (21). It was reported previously that wild *M. crassus* jirds from the Negev Desert (our study area) were infested with a mean of 12.56 fleas (usually mixed flea species, dominated by *X. ramesis*) per jird (20).

By continuously culturing and counting the numbers of *Bartonella* CFU in the blood of the experimentally infected jirds, we noticed that the jirds in this study were persistently infected for periods of 100 to 161 days. Moreover, bacteremia levels increased rapidly in the first 18 days after subcutaneous inoculation of *Bartonella* or infestation with infected fleas, remained relatively high for an additional 90 to 100 days, and decreased thereafter until the disappearance of infection (Fig. 2A and B). *Bartonella* CFU numbers in jirds varied within the same jird and between jirds, ranging from 20 to more than 1,000 CFU per 200 μl of blood during the peak of bacteremia. A previous study reported that *Bartonella coopersplainensis*-like (strain Rn1691yn)-infected Swiss Webster mice produced bacteremia at levels as high as 10^5 bacteria/ml of blood that lasted from 3 to 8 weeks (22). In our study, a longer duration of bacteremia was recorded (up to 26 weeks), suggesting a better evolutionary adaptation of *Bartonella* sp. OE 1-1 to *M. crassus* jirds than that of the rat-associated *B. coopersplainensis*-like strain to Swiss Webster mice.

The kinetics of *Bartonella* sp. OE 1-1 bacteremia in the subcutaneously injected jirds resembled that of the flea-infested jirds. Pronounced fluctuations in the CFU counts could be noticed, suggesting a possible cyclic bacteremia in naturally infected jirds. Cyclic bacteremias in laboratory and wild-captured *Bartonella*-infected rodents have been described (12, 23). In an experimental study, bacteremia onset was shown to occur several days after inoculation by a synchronous wave of bacterial invasion into mature erythrocytes, followed by intracellular bacterial replication until a stagnant number was reached and sustained for the remaining life span of the infected erythrocyte. The initial wave of erythrocyte infection was followed by reinfection waves occurring at intervals of several days. It was proposed as a bacterial persistence strategy, adapted to a nonhemolytic intracellular colonization of erythrocytes, that permits blood-sucking arthropods to efficiently transmit the bacteria (1).

The occurrence of maternal transmission of *Bartonella* from the mother jird to its offspring was also investigated in this study. Blood cultures of the jird offspring were all found to be negative for *Bartonella*. However, *Bartonella* sp. OE 1-1 DNA was detected in 1 spleen sample from a pup of a *Bartonella*-positive female. Our results indicate that maternal transmission from a female jird to its offspring can occur, although whether the route is transplacental, via the milk, via saliva, or by bites cannot be concluded from the current study and have to be further investigated. It seems that even if maternal transmission of this *Bartonella* strain exists in

jirds, it is not a major route of transmission, and as such, a noneffective route would be in disagreement with the high prevalence of this *Bartonella* strain in the Negev jirds (10). The results of the current study suggest that fleas probably have a major role in maintaining *Bartonella* sp. OE 1-1 among *M. crassus* jirds in nature. No evidence of either horizontal or maternal transmission was found in bank voles inoculated with *B. taylorii* maintained in an arthropod-free environment (3). An absence of maternal transmission was also reported in cats experimentally infected with *B. henselae* (24, 25). On the other hand, *Bartonella* could be isolated from neonates and embryos of naturally infected wild-captured North American cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) (11). In another study, transplacental transmission of *Bartonella* was demonstrated when 76% of the fetal resorptions in BALB/c mice were found to be culture positive for *B. birtlesii*, although none of the viable offspring was found to be bacteremic (12). The contradicting results on maternal transmission of *Bartonella* in rodents in several studies conducted to date might suggest different transmission patterns of different *Bartonella* spp. in different rodent species.

The same number of offspring was delivered by both *Bartonella*-infected and *Bartonella*-free female jirds. However, when the sex ratios of the offspring were compared, more male newborn jirds were observed in the *Bartonella*-positive group than in the *Bartonella*-negative group (12 males and 3 females in the former versus 8 males and 5 females in the latter). Although the differences were not significant, they might suggest a possible effect of *Bartonella* infection on *M. crassus* newborn jirds, such as a higher mortality rate for female embryos. It has been reported that *B. birtlesii* infection in BALB/c mice affected the outcome of pregnancy, causing resorption, fetal death, and fetal weight loss (12). In order to test the possible occurrence of this effect, a larger group of female jirds would have to be included and compared in future studies.

Bartonella sp. OE 1-1 and *B. elizabethae* show 10 bp differences between their 313-bp *gltA* gene fragments, suggesting that they are closely related and supporting the inclusion of the former in the *B. elizabethae* species complex, as previously suggested (26). As *B. elizabethae* is a confirmed zoonotic species (27), the zoonotic potential of *Bartonella* sp. OE 1-1 has to be further investigated. During this study, no observable clinical abnormalities were noticed in any of the infected jirds. Future studies, including gross pathology and histopathology, are required to assess whether this *Bartonella* strain is virulent for jirds and other rodents.

In conclusion, this study demonstrates that *X. ramesis* fleas are competent vectors of *Bartonella* sp. OE 1-1 (a strain closely related to the zoonotic *B. elizabethae*) to *M. crassus* jirds. Maternal transmission of *Bartonella* sp. OE 1-1 from female jirds to their offspring seems to be feasible but is not a major transmission route. The current results suggest that *X. ramesis* fleas are probably playing an essential role in maintaining this *Bartonella* strain in the jird population in nature.

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