

TICK-BORNE RELAPSING FEVER (RF) IN THE CIVILIAN POPULATION OF ISRAEL, 1980 - 2002

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Abstract Tick-borne relapsing fever (RF) is considered to be caused by the spirochaete *Borrelia persica*, transmitted to humans by *Ornithodoros tholozani*, and is a notifiable disease in Israel. Between 1980 and 2002, 184 cases were reported, i.e. an average of eight cases per year (range 0-16). Among them, about 50% were children and more than 80% were day-trippers. The infestation foci were principally caves. Many of the foci are sites in, or near the periphery of, urban areas where it is easy for people to visit them. Professional visitors working in caves were rarely infected, since they generally take preventive measures against tick bites. The distribution of the disease is all over the country except the southern Negev Desert. This also indicates the distribution of *O. tholozani*. Tick samples were collected in several locations from which clinical cases were reported. Partial sequences of the 18S rDNA were amplified by PCR and sequenced. The analysis of a 600 bp fragment by blast gave 84% homology with *Ornithodoros moubata*. DNA extracts of a pool of ticks and of patients' blood samples were successfully amplified and produced an approximately 780 bp fragment of the flagellin gene of *Borrelia*. Sequence similarity was more than 99% with RF strain species and 85% with Lyme borreliosis species. Taxonomic analysis showed that sequences of the Israeli RF *Borrelia* clustered in a separate group from the American and the African RF species. This is the first report of molecular characterization of the etiologic agent of RF and its tick vector in the Middle East. The exact taxonomic link to the previously described *B. persica* requires further study.

Key Words Tick-borne relapsing fever, *Borrelia persica*, *Ornithodoros tholozani*

INTRODUCTION

Tick-borne relapsing fever (RF) is caused by spirochaete species of the genus *Borrelia*, and is transmitted to humans by soft ticks of the species *Ornithodoros* (Argasidae). Each species of *Borrelia* is transmitted within the geographical range of a particular species of *Ornithodoros* vectors (co-speciation concept), for example, *B. duttonii* is transmitted by *O. moubata* in eastern and western Africa.

In Israel, RF (cave fever) is considered to be caused by the spirochaete *Borrelia persica* and is transmitted to humans by the cave tick *Ornithodoros tholozani*. This tick and the disease are distributed in Central Asia (Kazakhstan, Kyrgyzia, Iran, Tajikistan, Turkmenistan, Uzbekistan, Afghanistan, India and Kashmir) and the Middle East (Iraq, Jordan, Syria, Lebanon, Israel, Egypt and Libya) (Calra and Roa, 1951; Parola and Raoult, 2001).

RF begins abruptly with chills, headache, myalgia and arthralgia after an incubation period of approximately 7 days following the tick bite. The primary attack lasts 1-5 days and the relapse occurs at irregular intervals. Usually three to five relapses are seen in cases of untreated RF.

In Israel, *O. tholozani* (Figure 1) inhabits caves, ancient ruins, archaeological sites etc. It digs itself into the soil up to a depth of 1 meter or hides itself in wall crevices where it finds suitable microclimatic conditions: very high relative humidity of 70-80 %, a relatively low temperature of 17-25° C and dim light conditions (Lidror, 1964; Avivi, 1967). In the northern countries of its distribution area, e.g., Iran, Kashmir, Afghanistan and the southern countries of the former Soviet Union, *O. tholozani* also lives in houses and cowsheds where the same suitable conditions can be found (Abidov et al., 1993; Arshi et al., 2002).

O. tholozani attacks any warm-blooded host which comes into its habitat range (Costa, 1978; Parola and Raoult, 2001). While an infected tick is sucking blood, the host becomes infected with *B. persica*. In various parts of the world, the reservoir of the *Borrelia* species are mainly rodents and small mammals and the *Borreliae* may persist for many years in their long-lived tick vectors (Parola and Raoult, 2001). In Jordan, one specimen of bat, *Pipistrellus kuhli*, was found to be infected with *B. persica* (Zulueta et al., 1971). In Israel there is no

information about the natural reservoir of this spirochaete. The only wild mammal found to be infected with *B. persica* in Israel is the badger, *Meles meles* (Costa, 1978). Despite this, it is known that there is transovarial transmission from the infected eggs to the next generations (Barbour and Hayes, 1986). Under these conditions, the vector is also the reservoir and RF in Israel is not a characteristic zoonotic disease, in which the causative agent is transferred from a reservoir animal.

The populations of *O. tholozani* in Israel are isolated from each other. This tick does not migrate from its habitat even under starvation conditions; only rarely is it dispersed while attached to a host, since the duration of the blood meal is only about 30 minutes. The tick can survive starvation for several years until a host passes within reach. This biological characteristic of *O. tholozani* explains the characteristic of relapsing fever which depends on human behaviour, i.e., whoever comes within range of the tick exposes himself to attack. The distribution of *O. tholozani* overlaps that of the disease. Several older surveys on RF were carried out between 1947 and 1981. The present work summarizes the epidemiological data of the disease from 1982 until 2002. One of the aims of this work is to try to map the infested areas according to the localities where the infections occurred.

Since the populations of ticks have been isolated from each other for a very long period, a question arises concerning their taxonomic position within the species *O. tholozani*. Here we have a first report of preliminary investigations of specimens of *O. tholozani* from Israel regarding their taxonomic position based on molecular biology. The continuation of these tests will enable the very important determination concerning the taxonomic position of all the Israeli *Ornithodoros* populations, which is a necessary basis for finding a method to isolate the spirochaetes from the tick and identify them on the basis of molecular biology.

The identification of the causative agent has been carried out till now on the blood of infected people using microscopical morphology on thin or thick blood smears. This is the first report of characterization of *B. persica* from *O. tholozani* and from patient samples using molecular biological methods.

MATERIALS AND METHODS

Epidemiological Data

Since RF is a notifiable disease, the doctor's report is transferred to the Regional Medical Service where the patient lives or is being treated. This office transfers all the reports to the Department of Epidemiology of the Ministry of Health. All reports from 1980 - 2002 were checked. The reports are in the form of a questionnaire which includes personal information of the patient, information about the disease, diagnosis, clinical symptoms, laboratory results etc. as well as epidemiological data. Sometimes, other documents are added such as hospital discharge papers and additional epidemiological enquiries. This work does not focus on the clinical aspects of the disease but rather on the epidemiological data: the number of cases of relapsing fever per annum; the source of infestation: the geographical area which is necessary for mapping of the infested area; the character of the infested place, e.g., caves, ruins; sex and age of the patient. In the survey of Schwartz (1970) there was a division into several age-groups. In this work it was decided to divide the ages of the patients into only two groups: up to 18 (children and youth) and 19 and over (adults). Occupation is also relevant. There is no meaningful significance of the occupation of the RF patients except the reasons why they were staying in the infested place. Therefore, the patients were divided into two groups: (i) those who penetrated into the ticks' living range because of hikes and visits to caves etc., and (ii) those who came into this range through professional reasons such as archeologists and zoologists. The month or season of infection is also relevant.

In all the reports from the Epidemiological Department, apart from a very few cases, there is a laboratory diagnosis identifying the causative agent of the disease, *B. persica*, using a blood smear. The laboratory diagnosis is in addition to the clinical diagnosis by the physician. Apart from the epidemiological diagnosis of the data, which indicates where the patient was staying in a characteristic focus, there is sometimes also a report concerning tick bites.

Bio-molecular Methods

Tick samples were collected in several locations from which clinical cases were reported. Fresh ticks were identified as *O. tholozani* at the Laboratory of Entomology, Ministry of Health and frozen at -80°C until use. Blood samples were tested from patients for whom the diagnosis was made on the basis of thick and/or thin blood smears and clinical picture. For DNA extraction from ticks, we used the Dneasy tissue kit of Qiagen (Cat. No. 69 504), samples were adjusted to 1nanog/microL. By this method, the extracted DNA from frozen ticks was of good quality. For blood samples from patients or mice, the QiAamp DNA Blood Mini Kit (Cat. No. 51 1040) was used on frozen whole blood samples.

PCR Methods

For ticks, The 18S rDNA tick gene was chosen for two reasons. The first was in order to design a PCR for *O. tholozani* to serve as an internal control to check the absence of PCR inhibitors in parallel with specific PCR assays for *Borrelia*. The second is because this gene has proven itself to be the most efficient for molecular taxonomic studies on ticks.

For *Borrelia*: At least, two genes were targeted with success. The first is the flagellin gene and the second is the 16S rDNA gene.

For flagellin we designed several new pairs of primers (BG1-BG2, BR1-BR2, BL1-BL2) able to amplify respectively, all *Borrelia* species, only RF species and only Lyme borreliosis species. We have also used previously published primers (Fukunaga, 1996; Fukunaga, 2001; Picken, 1992 and Ras, 1996).

The PCR assay was checked on one representative strain of the RF group (*B. duttonii*) and one representative strain of *Borrelia* associated with Lyme borreliosis (*B. burgdorferi* sensu stricto, strain B31). These two DNA strain extracts were strongly positive as expected. PCR products were purified by Promega kit wizard PCR prep and cloned in T7 plasmid by pGEM-T Easy vector SystemII of Promega, according to the manufacturer's instructions. Plasmids containing inserts were purified by Qiagen Qiaprep Spin Miniprep kit (Cat. No 27 104) and sent to Danyel Biotech Ltd (Rehovot, Israel) for two strands sequencing.

Sequence and Taxonomic analysis

DNA consensus sequences were blasted at <http://www.ncbi.nlm.nih.gov/> with All GenBank+EMBL+DDBJ+PDB. ClustalW and Njplot software were used for alignment of the sequences and tree constructions.

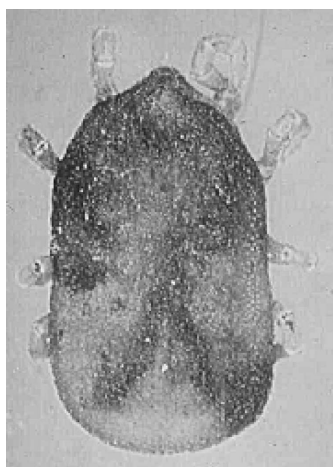


Figure 1. *Ornithodoros tholozani*

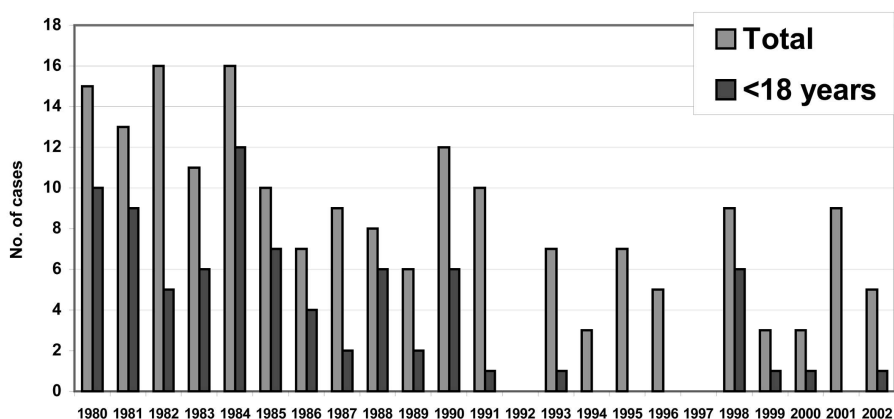


Figure 2. RF cases in Israel, 1980-2002

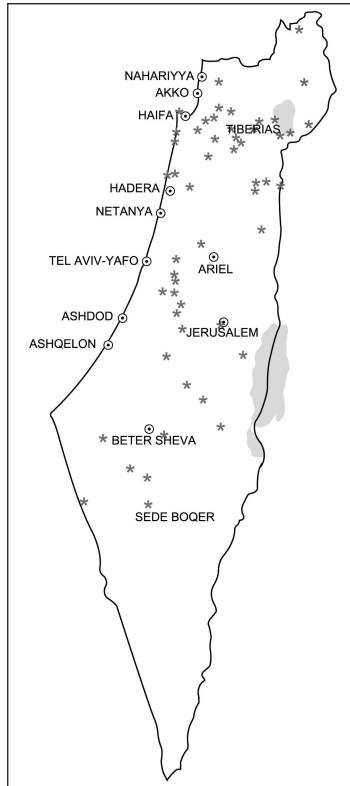


Figure 3. Infection foci of RF, 1980-2002

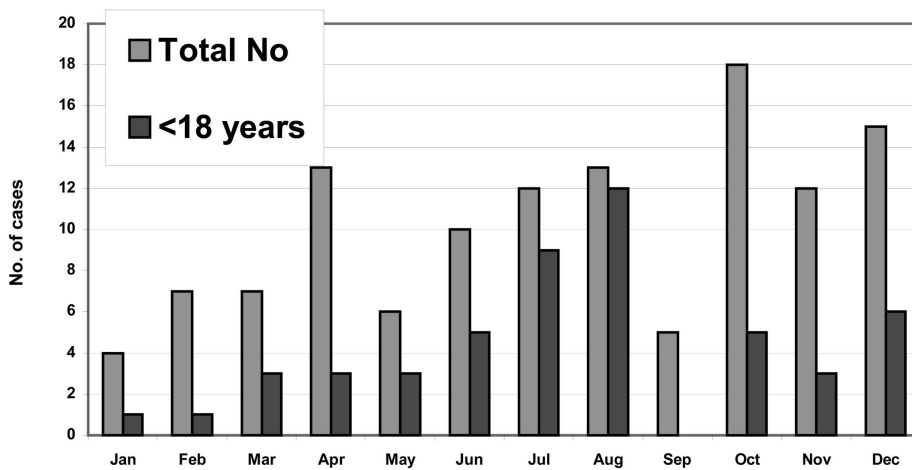


Figure 4. Monthly distribution of RF cases, 1980-2002

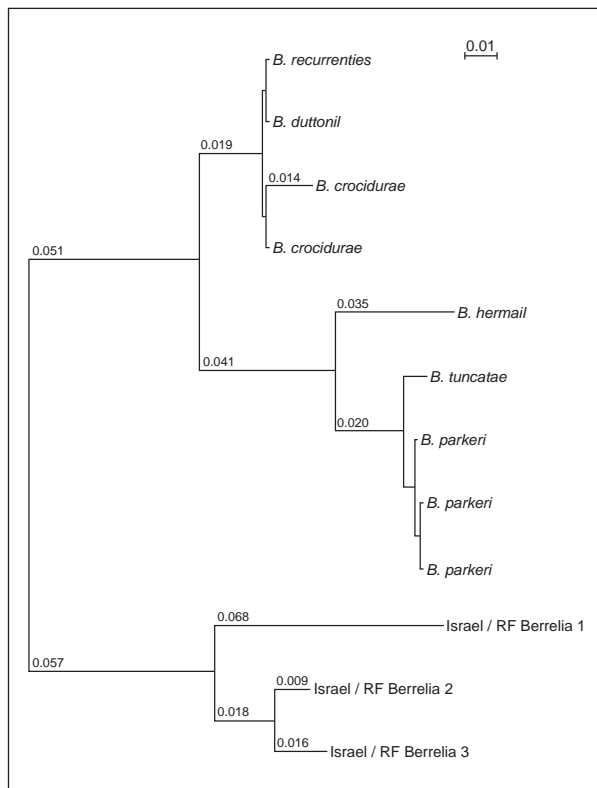


Figure 5. Phylogenetic tree based on comparison of the flagellin sequences of RF *Borrelia* species



Figure 6. *Ornithodoros coniceps*

RESULTS

Epidemiological data

During the years 1980 and 2002, 184 cases of RF were reported, an average of 8 cases per year, range 0-16 (Figure 2). However, the average number of cases gradually decreased from 14.2 between 1980 and 1984, to 8.8 between 1985 and 1991, to 4.6 between 1992 and 2002. No fatal cases were recorded between 1980 and 2002, nor in earlier surveys. In 2003, eight cases of RF were recorded and five in 2004.

Table 1. The incidence of RF cases from 1947 to 2002

Years	Population in millions	No. of cases per year	Source of information
1947	0.6	97	Yekutiel, 1956
1950-54	1.32 – 1.5	13-55	Kochavi, 1960; Yekutiel, 1956
1955-60	1.55 – 2.11	25-59	Shwartz, 1970
1961-66	2.19 – 2.56	4-26	Shwartz, 1970
1967	2.72	42	Shwartz, 1970
1971-81	3.07 – 3.95	92	Dept. of Epidemiology, MoH
1980-1991	3.88 – 4.95	6-16	Present survey
1992-2002	5.12 – 6.57	0-9	“ “

Table 2. Analysis of persons infected with RF, 1980-2002

Youth, up to 18 years		
1980-2002	48.4%	girls 40%, boys 46%
1991-2002	18%	
Adults	50.9%	women 23%, men 70%
Minorities	9 cases	women 4, men 5
1954-1967	241 cases	
Hikers	92.4%	
Professionals	7.5%	

A number of older sources of RF data were used in order to compare the number of cases and the population levels in Israel between 1947 and 2002 (Table 1).

In Table 2, an analysis of the data from the epidemiological reports (1980-2002) is given, showing the relative numbers of youth, adults, minorities and professions amongst the infected persons.

The distribution of RF infestation foci in Israel is shown in Figure 3. The southernmost focus of RF in Israel was recorded from Sede Boker in the northern Negev Desert. The 75 infestation foci were characterized as follows: caves, 88%, ruins, 5%. Several cases were also recorded from the following places: ancient fortress; ancient water cistern, old cemetery, porcupine burrow and a niche under a rock. Figure 4 shows the monthly distribution of RF cases. 75% of people infected with RF in July and 92% in August were young people under 18 years old. July and August is the long summer vacation time in Israel.

PCR of *O. tholozani*,

A collection of about 200 ticks from 5 different areas were stocked for the first year of the study in order to develop the bio-molecular methods. Tick DNA extracts were first tested with published primers for 16S mitochondrial rDNA gene with no success (Black, 1994). Further attempts were made with other published primers (NS1, NS2, NS4, NS8) designed to amplify the 18S rDNA tick gene (Black, 1997). PCR for NS1-NS2 and NS1-NS4 were positive and generated 600 and 1200 bp fragments respectively. The sequence of the 600 bp amplicon was performed. The analysis of this fragment by blast gave 84% homology with *Ornithodoros moubata*.

PCR of *Borrelia* species

PCR attempts with DNA extracts from individual ticks were negative, but were positive with pools from 10 ticks. These extracts were positive for the flagellin target (BG1-BG2) and for the 16S rRNA, with an expected fragment of respectively 770 and 600 bp.

For the flagellin gene target (BG1-BG2), DNA extracts from 7 patient samples were positive by PCR. The mouse blood sample infected with patient blood was also positive. Four different amplicons were successfully cloned and sequenced and gave an approx. 780 bp long fragment. DNA consensus sequences gave more than 99% identity with RF strain sequences (*B. duttonii* and *B. crocidurae*) and 85% with Lyme borreliosis associated strains.

Tree construction for taxonomic analysis showed that sequences of the Israel RF *Borrelia* clustered in a separated group (Middle East RF species) from the American and the African RF species (Figure 5).

DISCUSSION

The incidence of RF cases depends on the behavior of the population. Despite the fact that there has been a constant and rapid increase in the population since the beginning of the 1950s, there has been a constant decrease in the number of RF cases (Table 1). The main reasons for the decrease in the number of cases are changes in behavior of the population, control operations in caves and tourist sites, and ecological changes.

Changes in behavior of the population which include (1) the awareness of hikers and their guides to the dangers of entering infested or unknown caves; (2) staying for only a very short time in the cave, without sitting or lying down; (3) personal prevention measures, such as the use of repellants and suitable clothing. Ecological changes include the prominent expansion of urban areas which are ruining caves sites which were serious foci for RF infestation.

The changes in the lifestyle of the minorities in Israel caused the drastic decrease in the number of cases among this population from 241 cases between 1954-1967 (Shwartz, 1970) to only 9 cases in 1980. These changes were mainly the changeover of the Bedouins to a settled way of life from a nomadic one, in which caves were used for sheltering flocks and other purposes. The percentage of cases among the youth decreased since the beginning of the 1990s from 80% of all cases to 18%. Naturally, in the summer holiday of July and August, most of the infested cases are among the youth. Only a few of the professionals who penetrate into the range of *O. tholozani*, such as archaeologists, contract the disease, since they are generally aware of the dangers and take preventative measures against the ticks, and control measures are taken before they begin working in such places.

Nevertheless, there are several reasons to argue that a significant percentage of cases are not declared. A lot of patients, particularly children, are examined by physicians at hospital emergency rooms for high fever. In some cases, they have been treated with antibiotics after blood sampling for blood cultures, that obviously returned negative. But because the high sensitivity of the bacteria to antibiotics, the diagnosis of RF remains undone. It is worth noting here that since the clinicians became aware that PCR for *Borrelia* diagnosis is performed in our laboratory, and that this method is more sensitive than the classical smears, we have received blood samples from more than 15 suspected cases of RF in 2004.

The distribution of RF is all over Israel as far south as Sede Boker in the northern Negev Desert. This distribution overlaps the distribution range of *O. tholozani*. It was found that 10% of 500 caves all over Israel were infested with *O. tholozani*. Caves in northern Israel and the coastal plain had an infestation rate of 30-60%, while in the semi-desert and desert areas, the infestation dropped significantly (Avivi, 1973). This tick is not found in the southern Negev Desert where the very high temperature and dryness are too severe for the ticks to survive.

Caves are the main foci for the distribution of *O. tholozani*, but a few cases were from ruins. Preventative control and extermination measures have been carried out at ancient fortresses and archeological sites which were investigated and opened to the public. Mammals may transfer the ticks to their burrows or others habitats where they may hide, so these places are also potential foci for infection. *O. tholozani* is active throughout the year (Avivi, 1967) therefore infection is also possible all through the year.

In 1954, Babudieri found that the vector of RF in the city of Nablus and its surroundings (north of Jerusalem) was a rare tick, *Ornithodoros coniceps*. This tick attacks man and it has been proved that it is a carrier of *Borrelia sp.* In 1997, a large population of *O. coniceps* was found in the center of Jerusalem, attacking a whole family (Wilamowski et al., 1999).

It is worthwhile emphasizing that in several of the documents concerning RF, *Borrelia recurrentis* is recorded as the causative agent. This identification is a mistake. *B. recurrentis* is the causative agent of epidemic relapsing fever, the vector of which is the body louse, *Pedulus humanus corporis*. Since 1946, there have been no reports of epidemic relapsing fever in Israel and neither has this species of body louse been found (Shwartz, 1970). This is also confirmed by our laboratory data and surveys.

According to the co-speciation concept, the *Borrelia* strains found in *O. tholozani* and in patients with RF are *B. persica*. However, we did not find sequences of the flagellin gene of this species in DNA-banks, but only the 16S rDNA sequence (Ras, 1996). We decided to work on the flagellin gene and not on the 16S rDNA gene because the latter is much less informative at the species level and is now considered by taxonomic specialists to be relevant only at the genus level. We also found the same flagellin sequence (780 bp) from patient and tick isolates. This fact allows us to establish that the *Borrelia* we have characterized is the causative agent of Israeli RF. All sequences are very similar to one another and cluster in a separate root in the tree analysis, but more samples are also necessary from ticks and patients in order to assess the level of molecular polymorphism of this species.

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REFERENCES CITED

- Abidov, Z.I., Vasil'eva, I.S. and Rakhimov, N.R. 1993. Tick-borne relapsing fever morbidity in Namangan. Med. Parazitol. (Mosk.) 1: 32-35 (in Russian).
- Arshi, S., Majidpoor, A., Sadeghi, H., Asmar, M., Emdadi, D. and Derakhshan, M.H. 2002. Relapsing fever in Ardabil, a northwestern province of Iran. Arch. Iran. Med. 5: 141-145.
- Avivi, A. 1967. Biology of the relapsing fever tick *Ornithodoros tholozani* (Laboulbene & Megnin, 1882) in Israel. WHO/VBC/68.57, p.85-90, publ. 1968. WHO Seminar on the Ecology, Biology and Control of Ticks and Mites of Public Health Importance. Geneva, Dec. 1967.
- Avivi, A., Warburg, M. and Galun, R. 1973. Ecological studies on the cave tick *Ornithodoros tholozani* and its distribution in Israel. Israel J. Entomol. 8: 109-129.
- Babudieri, B. 1955. Survey on relapsing fever in Jordan, July-September 1954, final report. WHO EM/Epid./3. Add. 1. 25 March 1955.
- Barbour, A.G. and Hayes, S.F. 1986. Biology of *Borrelia* species. Microbiol Rev. 50: 381-400.
- Black, W.C. 4th and Piesman, J. 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc. Natl. Acad. Sci. USA 91:10034-10038.
- Black, W. C. 4th, Klompen, J.S., Keirans, J.E., Piesman, J., A. J. Mangold, A.J., Bargues, M.D. and Mas-Coma, S. 1997. Phylogenetic relationships among tick subfamilies (Ixodida: Ixodidae: Argasidae) based on the 18S nuclear rDNA gene. Mol. Phylogene. Evol. 7:129-144.
- Costa, M. 1978. Insects anti Man. Israel: Hakibbutz Hameuchad (in Hebrew).
- Fukunaga, M., Okada, K., Nakao, M., Konishi, T. and Sato, Y. 1996. Phylogenetic analysis of *Borrelia* species based on flagellin gene sequences and its application for molecular typing of Lyme disease borreliae. Int. J. Syst. Bacteriol. 46: 898-905.
- Fukunaga, M., Ushijima, Y., Aoki, L.Y. and Talbert, A. 2001. Detection of *Borrelia duttonii*, a tick-borne relapsing fever agent in central Tanzania, within ticks by flagellin gene-based nested polymerase chain reaction. Vector Borne Zoonotic Dis. 1: 331-338.
- Kalra, S.L. and Rao, K.N.A. 1951. Observation on the epidemiology of the relapsing-fever in Kashmir. Indian J. Med. Res. 39: 3.
- Kochavi, Z. 1960. Relapsing fever transferred by ticks, national summary, 1953-1958. Briut HaTzibur, 3: 24-29 (in Hebrew).
- Lidror, R. 1964. Relapsing fever tick, ways of prevention and extermination. Tavruah, 24: 26-30 (in Hebrew).
- Parola, P. and Raoult, D. 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. 32: 897-928.

- Picken, R.N. 1992.** Polymerase chain reaction primers and probes derived from flagellin gene sequences for specific detection of the agents of Lyme disease and North American relapsing fever. *J. Clin. Microbiol.* 30: 99-114.
- Ras, N.M., Lascola, B., Postic, D., Cutler, S.J., Rodhain, F., Baranton, G. and Raoult, D. 1996.** Phylogenesis of relapsing fever *Borrelia* spp. *Int. J. Syst. Bacteriol.* 46: 859-865.
- Shwartz, T.A. 1970.** Endemic relapsing fever in Israel 1954-1967. M.P.H. Thesis, Hebrew University, Jerusalem, 106 pp. (in Hebrew, English summary).
- Wilamowski, A., Bromley-Schnur, H.J., Ioffe-Uspensky, I. and Uspensky I. 1999.** Ticks (Ixodoidea) in Israeli towns. *Proc. 3rd Internat. Conf. Urban Pests 1999:* 477-483.
- Yekutiel, P. 1956.** Epidemiology of insect-borne diseases in Israel. *Tavruah* 8: 8-14 (in Hebrew; English text in special issue, pp. 5-12).
- Zulueta de, J., Nasrallah, S., Karam, J.S., Anani, A.R., Sweatman, G.K. and Muir, D.A. 1971.** Finding of tick-borne relapsing fever in Jordan by the malaria eradication service. *Ann. Trop. Med. Parasitol.* 65: 491-495.