EPIDEMIOLOGICAL STUDY ON TICK-BORNE (ACARI: ARGASIDAE) RELAPSING FEVER IN KURDISTAN PROVINCE, IRAN, 2000-2004

Javad Rafinejad[1,2], Khadijeh Shemshad[3,*] and Omid Banafshi[4,*]
[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2]Evaluation Management and Development Center, Deputy of Research Ministry of Health and Medical Education, Tehran, Iran

[3]Department of Entomology, Science and Research Branch, Islamic Azad University, Tehran, Iran

[4]Kurdistan University of Medical Sciences, Sanandaj, Iran

*Corresponding Authors; E-mail: khadijehshemshad@gmail.com; omidbanafshi@yahoo.com

Abstract

Tick-borne relapsing fever is an acute infectious disease transmitted to humans by *Ornithodoros tholozani*, and it is a notifiable disease in Kurdistan Province, Iran. This cross-sectional survey was carried out from 2000 to 2004. The main aim of this study was to ascertain the prevalence of tick-borne relapsing fever in Kurdistan Province. The prevalence and incidence of tick-borne relapsing fever was monitored by daily clinical surveillance and by thin and thick blood smears of individuals with a fever. In confirmed cases, there was febrile illness, and spirochetes were identified on smears of peripheral blood. A field survey on presence of *Ornithodoros tholozani* in Bijar county villages was carried out and investigated for the detection of *Borrelia* spp. A total number of 97 cases including 88 cases from rural areas and 9 cases from urban areas were recorded over 5 years. Epidemiological studies on *O. tholozani* ticks collected from the several locations of villages indicated that 2 of the 20 studied villages (10%) were infested by the vector tick, *O. tholozani*. The presence of *O. tholozani* in most villages investigated and its infection rate suggest that tick-borne relapsing fever is a common cause of fever in most rural areas of Kurdistan Province. Results of the study demonstrate that tick-borne relapsing fever is under-recognized and under-reported, and the pathogens may be wrongly identified as malaria parasites. The study showed that tick-borne relapsing fever should be considered as an important public health priority in the study area.

Key Words: tick-borne relapsing fever; *Borrelia persica*; *Ornithodoros tholozani*; Kurdistan

Resumen

La fiebre recurrente es una enfermedad aguda infecciosa transmitida por las garrapatas *Ornithodoros tholozani* a los humanos, es considerada una enfermedad de declaración obligatoria en la provincia de Kurdistán, Irán. Se realizó esta colecta a travez de la región desde 2000 hasta 2004. El objetivo principal de este estudio fue para determinar la prevalencia de la fiebre recurrente transmitida por garrapatas en la provincia de Kurdistán. Se monitoreó la prevalencia y la incidencia de fiebre recurrente transmitida por garrapatas por medio de la vigilancia clínica diaria y por manchas de sangre aguado y espeso de individuos con fiebre. En los casos confirmados, se presentó una enfermedad febril y las espiroquetas fueron identificadas en las manchas de sangre periférica. Se realizó un estudio de campo sobre la presencia de *Ornithodoros tholozani* en los pueblos de Bijar para evaluar la presencia de *Borrelia* spp. Se registraron un total de 97 casos, incluyendo 88 casos de las zonas rurales y 9 casos de las zonas urbanas durante 5 años. Los estudios epidemiológicos sobre las garrapatas *Ornithodoros tholozani* recolectadas de varios pueblos indican que 2 de los 20 pueblos estudiados (10%) estaban infestados por esta garrapata vector. La presencia de *Ornithodoros tholozani* en la mayoría de los pueblos investigados y su tasa de infección sugieren que la fiebre recurrente transmitida por estas garrapatas es una de las causas comunes de fiebre en la mayoría de las zonas rurales de la provincia de Kurdistán. Los resultados del estudio demuestran que la fiebre recurrente transmitida por las garrapatas no es bien reconocida y reportada y los agentes patógenos pueden ser erróneamente identificados como parásitos de la malaria. Los resultados del estudio, mostraron que la fiebre recurrente transmitida por garrapatas debe ser considerada como una prioridad de salud pública importante en el área de este estudio.

Palabras Clave: la fiebre recurrente transmitida por garrapatas, *Borrelia persica; Ornithodoros tholozani*; Kurdistan

Relapsing fever is a recurrent acute febrile infection caused by various *Borrelia* (Spirochaetales: Spirochaetaceae) spirochetes transmitted either by lice (*Pediculus* spp.) (epidemic relapsing fever) or by soft ticks (*Ornithodoros* spp.) (endemic relapsing fever) (Dworkin et al. 2002; Chaudhry et al. 2010). Tick-borne relapsing fever (TBRF) is a zoonotic disease involving many species of rodents and small mammals in widely distributed areas throughout the world. The *Borrelia* may persist for many years in their long-lived tick vectors (Gray et al. 2002). This disease was once a disease of global epidemic importance (Cutler et al. 2009) and is characterized by influenza-like symptoms. The endemic tick-borne relapsing fever spirochetes are transmitted by *Ornithodoros* ticks (Ixodida: Argasidae); *O. tholozani* serves as the principal vector for *Borrelia persica* in different parts of Iran. Acquisition of human TBRF is occasionally restricted to the geographical range of the soft tick vector's activity (Goubau 1984). Because soft ticks are associated with hosts for their typically rapid nocturnal feeding and are transient feeders with painless bites these factors limit the distribution of TBRF infection to the confines of the areas where tick vectors reside (Southern & Sanford 1969). In disease-endemic regions, diagnosis is typically carried out through demonstrating spirochetes in stained blood smears from patients or animal inoculation (Cutler et al. 2010). The onset of the disease is generally acute, with chills, high fever, intense frontal headache, and pains in the back, limbs, joints, and nausea and vomiting may occur (Gaud & Morgan 1945). The disease may be confused with influenza and malaria and is very common but sporadic in various parts of the world and in small clusters throughout rural areas in the northern temperate region of the world (Vial et al. 2006). *Borrelia* species vary their surface antigens, causing repeated stimulation of the immune system by each new antigen (Rodhain 1976; Trape et al. 1991). TBRF are reported from several areas located in central and southern Iran and its main foci are mountainous areas of west and north western of Iran. According to the Ministry of Health of Iran (unpublished documents), the total numbers of 201, 205 and 471 relapsing cases were reported in 2001, 2002 and 2003, respectively. The main aim of this study was to investigate the epidemiology of TBRF in Kurdistan Province.

Materials and Methods

Clinical Samples

Clinical samples consisted of blood smear samples of all patients who referred to health centers of Kurdistan Province from 2000 till 2004 with relapsing fever signs and longitudinal study was done. Relapsing fever is endemic to this region. For each patient at all studied sites, the presence or absence of a standard set of 13 clinical manifestations was recorded; demographic, clinical, and geographical data were obtained by interviewing patients and their illness history and potential risk factors for illness for the patients. A history of recurrent illness symptoms including fever, chills, sweats, myalgias, vomiting, myalgia, nervousness, recurrent fever, stomach ache, nose bleeding, photophobia, arthralagia, cough and head ache. A confirmed case was defined as a person who had both febrile illness and detection of spirochetes by Wright-Giemsa stained peripheral thin and thick blood smear microscopic investigation that was obtained from peripheral finger prick blood. The slides were examined under 100X objective using an oil immersion objective and dark field microscope. Two hundred oil-immersion microscope fields on each smear were examined.

Soft Tick Collection

Tick collections were done by 4 persons traveling to different villages in different topographic and geographical regions. For tick collections visits to villagers' houses, animal and poultry shelters were done by daylight in localities favorable to the nocturnal feeding habits of the soft tick vector, and people were randomly questioned regarding their geographic origin and the frequency of tick bites. All cracks, crevices, ceilings and floors of houses of 20 randomly selected villages were considered for tick collection and collected ticks were transferred into holding tubes. Tick collection time was about 20 min in each location and totally, 100 randomly selected locations were inspected for ticks. In each village, 5 houses were randomly selected, i.e., one each in the north, south, east, west and central part of the village. Soft tick aggregations were investigated in human and livestock dwelling including doghouses, barns, and poultry shelters. The field work was started in the spring as soon as weather conditions permitted. All specimens were identified on the basis of their morphological characteristics. In order to investigate *Ornithodoros tholozani* infection of *Borrelia* spirochetes, experiments were carried out in 3 different stages. Soft ticks were cleaned with 70% ethanol in order to minimize external contamination. Ticks were smashed in 4 mL of normal saline.

At the first stage, a suspension of 10 *O. tholozani* that had been collected in the winter were inoculated into 4 guinea pigs (0.25 mL each) and 8 laboratory mice (0.5 mL each). In the second stage, 26, O. *tholozani* out of 77 specimens were fed on a Guinea pig for 30-45 min. In stage three, 33 samples of *O. tholozani* ticks were smashed and then injected subcutaneously into 33 guinea pigs. One laboratory mouse and one Guinea pig

served as unexposed control animals. After 4 d to 2 wk, blood samples obtained from an infected Guinea pig were examined microscopically to determine the presence of *Borrelia* in their peripheral blood samples. Data were analyzed by using SPSS version 11.0.1 (SPSS Inc., Chicago, Illinois, USA).

Results

Frequency of Relapsing Fever during 2000-2004

The first report of the disease was in July 2000, and this case was a 24 yr old rural woman suspected of having malaria with fevers and chills. The numbers of TBRF cases recorded in successive years during 2000 to 2004 (Figs. 1 and 2) were as follows: 14, 8 18, 36 and 22, respectively.

Frequency of Disease Based on Geographical Region

Out of the 97 recorded cases of relapsing fever during 2000-2004, 92 were recorded from Bijar County, 2 from Qorveh County and 3 from Divandare County.

Frequency of Disease Based on Rural or Urban Areas

Out of 97 recorded cases of disease 88 (90.7%) of them occurred in rural areas and 9 (9.3%) of them were from urban areas, and most of the latter had travelled to rural areas in their medical history.

Frequency of Disease Based on Months, Seasons, Different Age Groups and Sexual Gender

In all years of the study, TBRF cases presented from Apr 2000 until Dec 2004 (Fig. 2). Sixty three cases were reported in the summer, and out of these 63 cases, 24 cases were reported in July, and the frequencies were high from July through Oct. Frequencies of this disease based on different age groups showed 42, 17, 17, 10, 5, 4 and 2 cases in 10-19 yr old, 20-29 yr old, 0-4 yr old, 5-9 yr old, 30-39 yr old, 40-49 yr old, and ≥50 yr old people, respectively. Incidence of the disease was 54.6% among men and 45.4% among women.

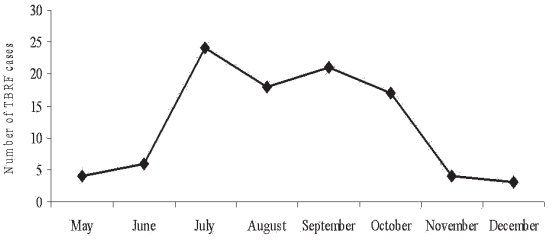

Fig. 2. Cases of tick-borne relapsing fever by month of onset during 2000-2004 in Kurdistan Province, Iran.

Frequency of Disease Based on Occupation

Out of the 97 cases of the disease, the occupations (Fig. 3) in 92 cases were students, farmers, housewives and children, and 5 diseased persons did not respond to this question. Among different occupations, students with 36 cases (37.1%) sustained the highest frequency of relapsing fever (Fig. 3).

Frequency of Disease Based on Demographic Characteristics and Kinds of Human Dwellings

The fractions of diseased people living in either in mud buildings or in the new bracket made buildings were 86.8% and 13.2%, respectively. More than 57% of the cases kept their livestock inside of the house yard, and those who had no livestock were living adjacent to neighbors who kept their livestock inside their homes. Seventy two percent of the patients were living within 10 m of the location of livestock. Twenty cases knew that they had been bitten by *O. tholozani*, but none of the bitten persons could distinguish between different tick species. Seventy seven cases either were not aware of having been bitten by a tick or were not able to respond to this question because of their age. Fifty one and one-half percent of the cases were living in crowded family settings of 6 or more persons in their family (Tables 1 and 2).

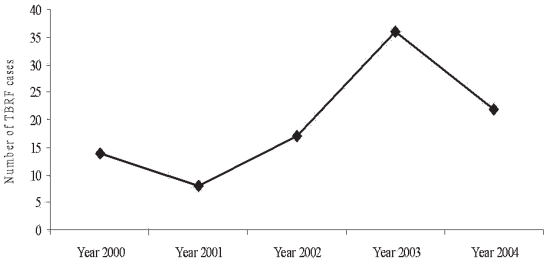

Fig. 1. Annual incidence tick-borne relapsing fever cases in Kurdistan Province, Iran during 2000-2004.

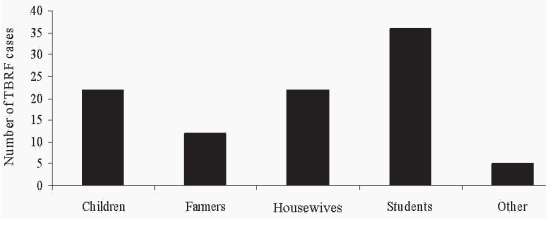

Fig. 3. Frequencies of tick-borne relapsing fever in Kurdistan Province, Iran based on patients' occupations

TABLE 1. FREQUENCIES OF TICK-BORNE RELAPSING FEVER CASES BASED ON NUMBER OF PERSONS IN THE FAMILY.

Number of persons in the family	Number of TBRF cases	Percentage of infected families
1-3	10	10.5
4-5	37	38
≥ 6	50	51.5

Clinical Signs of the Disease

One hundred percent of the patients have severe fever and 95% of them had fever and chills together. Results of the microscopic detection showed spirochetes in Wright-Giemsa stained blood smears from all patients. Seventy one percent of the patients had head aches and 90.4% had recurrent fevers (Fig. 4).

Results of Tick Collection and Determining *Borrelia* Infection

In order to determine distribution and seasonal activity of Argasidae ticks in the study areas, a tick survey was carried out from the winter of 2002 until December 2003. Tick collections were done by 4 persons traveling to different villages in different topographic and geographical regions. A total of 8,537 argasid ticks belonging to 2 genera were collected from Bijar County (Table 3). The percentages of *Argas* and *Ornithodoros* were 45.7% (n = 3904) and 54.3% (n = 4639), respectively. *Ornithodoros lahorensis* had the most frequency among the 4 identified argasid ticks. This species had the most frequency in 4 seasons. *Ornithodoros tholozani* and *Ornithodoros lahorensis* were collected from stables and huts. Eighty percent of the ticks were collected from mud made stables, 15% from huts, and 5% of them from newly constructed bracket stables. *Argas persicus* had the most frequency in poultry nests, but only few of the argasid ticks were collected from human dwellings (Table 3). In order to determine the relationship of *Ornithodoros tholozani* to *Borrelia persica* infection, investigations were carried out in 3 different stages, in winter 2002, spring 2003 and summer 2003. At the first stage in winter 2002, inoculations of 10 *Ornithodoros tholozani* to 4 guinea pigs and 8 laboratory mice were negative. The blood of the mice did not become spirochetemic during the 10 days after tick bite and all blood smears were microscopically negative for spirochetes. In the second phase, in spring 2003, 26 of the collected ticks were allowed to feed on laboratory mice and guinea pigs to determine whether they were infectious. Only one guinea pig showed infection, that sample belonged to GolBolagh village. In phase 3, in summer 2003, out of 392 live collected ticks, nearly 10% (n = 33) of them were selected randomly and their suspensions were inoculated intraperitoneally to guinea pigs. After 7 days, infections were recorded in one of the guinea pigs and the calculated rate of infection was 3%.

DISCUSSION

Tick-borne relapsing fever spirochetes were detected in Wright-stained blood smears from 97 patients. Samples of wild ticks were injected intraperitoneally into guinea pigs, and one guinea pig produced detectable levels of spirochetemia. All patients presented with raised temperatures, headaches, shivering and joint pains. *Ornithodoros* ticks may hide themselves in the cracks and crevices of mud or grass walls, and even in thatched roofs. The human dwelling was the preferred habitat for *Ornithodoros*. Regular nocturnal feeding by ticks could lead to efficient transmission of these spirochetes to humans (Cutler et al. 2010). TBRF occurred with the most frequency during summer months. However, TBRF also occurred during the winter. *Ornithodoros* species may lay dormant for several yr awaiting a blood meal, and some species do not require a blood meal from an infected host to harbor *Borrelia* (Cutler et al. 2010).

In most of the human dwellings, there were more than one species per dwelling, and *O. lahorensis* and *A. persicus* were seen together. But microhabitat/hiding place preferences among the species were expressed in a way so that *A. persicus* ticks were seen mostly in poultry shelters and *Ornithodoros tholozani and O. lahorensis* were seen in sheep barns in most cases. *Ornithodoros tholozani* were collected in 75% of the studied villages. This matter is very important because *Ornithodoros tholozani* lives in cracks in the ceilings of houses, and because of the proximity of live-

TABLE 2. FREQUENCIES OF TICK-BORNE RELAPSING FEVER BASED ON DISTANCE OF LIVESTOCK KEEPING LOCATION WITH HUMAN DWELLINGS.

Distance of livestock from human dwelling place (meters)	Number of TBRF cases	Percentage of infected families
1-2	10	13
3-5	24	31
6-10	22	28
≥ 10	22	28

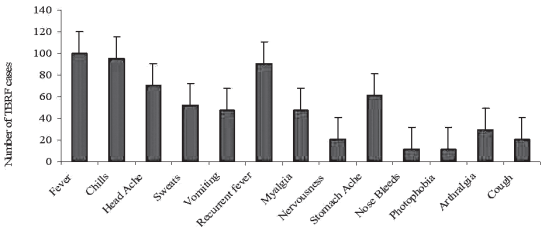

Fig. 4. Clinical symptoms in 97 patients with confirmed cases of tick-borne relapsing fever in Kurdistan Province, Iran.

stock keeping areas to human dwelling. Eighty, 15 and 5 percent of the collections were from mud barns, livestock dwellings made in mountains (places like caves that has been created by humans), and renovated barns, respectively. *Argas persicus* was collected mainly from poultry shelters. This species had the most frequency in the summer.

In order to determine the relationship of *Ornithodoros tholozani* to *Borrelia persica* infection, investigations were carried out in 3 different stages, in winter 2002, spring 2003 and summer 2003. The rates of *Borrelia persica* infections in students, housewives, farmers, children and other were 40%, 24.8%, 20.6% and 4.2% respectively. These differences in infection rates between occupations were not statistically significant ($\chi^2 = 1.502$, df = 4, $P = 0.826$), but these data show that all occupational groups are at risk of acquiring infection. Statistical analysis of the relationship of family size to infection rate showed that 51.5%, 37%, and 11.5% of the infections were observed in family sizes of ≥ 6, 4-5, and 1-3 persons in a family, respectively (Table 1). This finding was significant statistically ($\chi^2 = 16.075$, df= 2, $P = 0.000$). Results showed that families living 1-2 m from livestock dwellings had the lowest infection rate (Table 2). This result was statistically significant ($\chi^2 = 12.301$, df = 2, $P = 0.002$).

The winter occurrences of TBRF most often results from human activity that alters the behavior of the tick vector (Burgdorfer 1976). The diagnostic standard for TBRF is detection of spirochetes in peripheral blood smears. However meeting this standard is impeded by inexperience of microscopists, increased use of automated differential counts, and examination of blood in asymptomatic intervals when spirochetes are absent from the circulation or present in amount below the level of detection. Patients complain of headache, backache, muscle pain, arthralgia, and abdominal pain (Cutler et al. 2010). TBRF is a serious, sometimes fatal illness, mainly among immunocompromised patients. Most of the huts in the villages were infested by hundreds of ticks. The epidemiology of TBRF is determined by the prevalence of tick vectors and the number of infected ticks (Cutler et al. 2010). The painless, nocturnal, and transient feeding of *Ornithodoros* probably explains why so few persons reported a history of a tick or insect bite (Southern & Sanford 1969). Clinicians should be informed on the importance of a detailed travel history and inclusion of TBRF in the differential diagnosis of febrile illness occurring during the spring and summer months in patients staying overnight in mud huts. This is important because some of the cases with illnesses diagnosed with TBRF were travelers from other provinces to endemic states. In the case of TBRF, appropriate countermeasures may include prevention and education of the public and physicians about the epidemiology and clinical presentation of the disease. Results of this study showed that TBRF should be considered a public health priority because foci of endemicity can persist for years and because ticks can remain infected with *Borrelia* species for several years, even in the absence of feeding, and can transmit infection either to their offspring or to their sexual partner during copulation (Heymann 2005). Results of the survey demonstrate that tick-borne relapsing fever is under recognized and underreported, and the *Borrelia* spirochetes may be falsely identified as malaria parasites. It is necessary to train the laboratory technicians for recognition of the spirochetes.

Table 3. Frequencies of various species of argasid ticks collected from human dwellings in various villages in Bijar county, Kurdistan province, Iran during successive seasons from the winter of 2002 through the autumn of 2003.

Season	No. of villages examined	*Ornithodoros tholozani*		*Ornithodoros lahorensis*		*Argas persicus*		*Argas reflexus*		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Winter 2002	10	10	1.3	481	64.6	254	34.1	0	0	745	14.9
Spring 2003	201	77	4	958	49	917	47	0	0	1952	19.5
Summer 2003	20	392	18.5	322	15.1	1404	66.2	4	0.2	2118	21.2
Autumn 2003	17	98	2.6	2301	61.75	1323	35.6	2	0.05	3722	43.7
Total	67	577	6.7	4062	47.6	3898	45.7	6	0.07	8537	25.4

Conflicts of Interest Statement

The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgments

We thank the Kurdish villagers for their continuous participation in the study; the staff of the Institute Pasteur of Iran and staffs of health centers of Kurdistan Province for their role in clinical data collection.

References Cited

Burgdorfer, W., 1976. The epidemiology of the relapsing fevers, pp. 191-200 *In* R. C. Johnson [ed.], The Biology of Parasitic Spirochetes. Academic Press, New York.

Chaudhry, M. T., Shaikh, E. A., and Subhana, A. 2010. First case report of louse—borne relapsing fever in Saudi Arabia. Ann. King Edward University 16(3): 220-222.

Cutler, S. J., Bonilla, E. M., and Singh, R. J. 2010. Population Structure of East African Relapsing Fever *Borrelia* spp. Emerging Infectious Diseases 16(7): 1076-1080.

Cutler, S. J., Abdissa, A., and Trape, J.-F. 2009. New concepts for the old challenges of African relapsing fever borreliosis. Clinical Microbiol. Infection 15: 400-406.

Dworkin, M. S., Shoemaker, P. C., Fritz, C. L., Dowell, M. E., and Anderson, D. E. 2002. The epidemiology of tick-borne relapsing fever in the United States. Am. J. Trop. Med. Hyg. 66(6): 753-758.

Gaud, M., and Morgan, M. T. 1945. Epidemiological study of relapsing fever in North Africa (1943-1945). Bull. World Health Org. 1(1): 69-92.

Goubau, P. F. 1984. Relapsing fever. A review. Ann. Soc. Belge. Med. Trop 64: 335-364.

Gray, J. S., Kahl, O., Lane, R. S., and Stanek, G. 2002. Lyme Borreliosis: Biology and control. CABI Publishing. 480 pp.

Heymann, D. L. [ed.]. 2005. Control of communicable diseases manual, 18th ed. Washington, DC, Amer. Public Health Assoc.

Rodhain, F. 1976. Borrelia et fievres recurrentes: aspects epidemiologiques actuels. Bull. Inst. Pasteur 74: 173-218.

Southern Jr., P. M., and Sanford, J. P. 1969. Relapsing fever: a clinical and microbiological review. Medicine 48: 129-149.

Trape, J. F., Duplantier, J. M., Bouganali, H., Godeluck, B., Legros, F., Cornet, J. P., and Camicas, J. L. 1991. Tick-borne borreliosis in West Africa. Lancet 337: 473-475.

Vial, L., Diatta, G., Tall, A., Ba, E. H., Bouganali, H., Durand, P., Sokhna, C., Rogier, C., Renaud, F. and Trape, J. 2006. Incidence of tick-borne relapsing fever in West Africa: longitudinal study. Lancet 368: 37-43.