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ORIGINAL ARTICLE

RELAPSING FEVER IN ARDABIL, A NORTHWESTERN PROVINCE OF IRAN

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Background– The main area of Iran affected by relapsing fever (RF) is Ardabil Province, for which *Borrelia persica* is the most common cause in this country. The aim of this study was to determine the epidemiologic characteristics of the disease, and the frequency of infection among ticks in this region.

Method– This clinical epidemiology and entomology study was performed on a total of 391 patients diagnosed with tickborne relapsing fever between 1998 to 2001. The presence of Borrelia with any species as well as the clinical characteristics were observed. *Borrelia* was identified in blood smears of 1,421 ticks collected from 130 indoor and 14 outdoor sites. The ticks were crushed and the suspension obtained was injected into the peritoneum of two mice and two guinea pigs to determine the frequency of infection among the ticks living in this region. Data including the tick species determination were collected through a questionnaire and analyzed using Chi-square and ANOVA tests.

Results– The most prevalent clinical manifestations were fever, chills and headache. Other findings included nausea, vomiting, sweating, abdominal pain, arthralgia, cough, photophobia, eosinophilia, hematuria, jaundice, petechiae and scleral congestion. Laboratory tests performed on 60 patients showed leukocytosis, high erythrocyte sedimentation rate (ESR) and anemia. Of the 1,421 ticks collected, 45.9%, 40.3% and 13.8% were of the *Ornithodoros lahorensisi*, *Ornithodoros tholozani* and *Argas persicus* species, respectively. The prevalence of ticks was highest in Khandabil village. The ticks collected from three villages were found to be infected with *Borrelia*.

Conclusion– This study is the first large-sized published report of tick-born RF until that time. The clinical manifestations were similar to those reported in other studies. Petechiae occurred less frequently in our study compared to louse-borne RF. The high frequency of anemia, which was not stated in most other reference articles, requires further investigation.

Keywords ● Ardabil ● *Borrelia* ●*Borrelia baltazardi* ●*Borrelia persica* ● Iran ● *Ornithodorus tick* ● *Ornithodorus tholozani* ● relapsing fever ● tickborne relapsing fever

Introduction

Relapsing fever (RF) is an acute infectious disease caused by arthroborne spirochetes of the genus *Borrelia*. It occurs throughout the world, except for a few areas in the Southwest Pacific, and is divided into tickborne and louseborne RF. The disease is characterized by recurrent episodes of fever and chills, which are related to spirochetemia. Tickborne RF, which was diagnosed microbiologically in the last century, is the most common type of RF in Iran. Among the four most common types of *Borrelia* in Iran, *B. persica* and *B. baltazardi* have been isolated from the blood of patients with RF. So far, *B. baltazardi* has been isolated from the blood of only one patient from Ardabil.[1] *B. persica* was first isolated from the blood of a patient from Ardabil by Dschunkowsky and Luhs in 1913 and this report has been used as a historical reference by later authors.[1,2] *B. persica*

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has a wide geographic distribution and is present mainly in East and West Azerbaijan and in the Alborz and Zagros mountain chains. Most *Borrelia* species are found in Northwest Iran, especially in Ardabil Province, and, during the last 2 years, 63.2% of all cases of RF reported in Iran were from this area.[3] The vector of *B. persica* is the tick *Ornithodoros*, which was first discovered in Iran and subsequently named *O. tholozani* by Tholozan in 1879.[4]

The aim of this study was to determine the epidemiologic and clinical characteristics of RF in Ardabil Province during the last 4 years, as well as the frequency and infection rate of *Ornithodoros* in Khalkhal district, the main disease area in Ardabil Province.

Materials and Methods

Clinical epidemiology and entomology of two types of tickborne RF were evaluated in this study. From 1998 to 2001, a total of 391 patients with a definitive diagnosis of RF in Ardabil Province were enrolled. Diagnosis was based on clinical manifestations as well as the presence of *Borrelia* under Giemsa staining in blood smears. Laboratory tests were also performed for 60 patients. The main laboratory tests were complete blood count, including RBC indices, hematocrit, hemoglobin and ESR.

We made an assumption that *Borrelia* was very prevalent in Khalkhal, thus chose this city and its villages for entomologic study. Thirteen villages (10% of all villages of Khalkhal) were randomly selected and specimens collected from indoor sites. One of the villages was selected at random and specimens were collected from outdoor areas and animal shelters. There were five designated areas in each village (North, South, East, West, and Center) and the specimens were collected from one human residence and one animal shelter within a specified hour. For outdoor sampling, two active rodent nests were targeted.

All ticks were transferred alive to the parasitology laboratory of the Pasteur Institute, Tehran, Iran. They were subsequently crushed and suspension was prepared by standard methods and each one injected into the peritoneum of two white mice and two guinea pigs, simultaneously. Peripheral blood smears were then prepared at regular intervals and examined for presence of Borrelia under light microscopy.[5]

Statistics and data analysis

A questionnaire designed by the Iranian Ministry of Health and Medical Education was used for data entry and the data were analyzed by the EPI info 2000 version 1.1.2 package (Centers for Disease Control and Prevention, Atlanta, GA, USA). Relative and absolute frequency tables were constructed and data was analyzed using ANOVA and Chi-square tests.

Results

Demographic Findings

A total of 391 (49 % females, 51% male) patients were studied. Most (84.3%) of the cases were children or people who did not work outside their homes. The mean ± SD age of the patients was 12.1 ± 11 years. Most patients (91.5%) were from Khalkhal or Kowsar; the remainder were from the cities of Nir, Germi or Ardabil. More than 84% of the patients were resident of the villages or had become infected during their stay in a village. In 70% of cases, the buildings they lived in were very old. Cattles or sheep were found in the homes of 64% of patients.

Clinical findings

The most common clinical manifestations were fever (93.3 %), followed by chills and headache (86.2 %, 85.9 %) (Table 1). Body temperature was recorded by physicians for 196 patients. Of whom 96.4% had body temperature higher than 38°C (39 ± 0.6°C mean ± SD). With the exception of chills, the other cardinal signs of RF were not different significantly between both sexes. Other clinical findings included nausea, vomiting, sweating, abdominal pain, arthralgia, cough, photophobia, eosinophilia, hematuria, icterus, petechiae and scleral congestion. The results of laboratory tests performed on 60 patients, showed leukocytosis, high erythrocyte sedimentation rate (ESR), and anemia. Chills were 10% more frequent in females than males and was statistically significant using Chi-square test ($\chi^2 = 7.8$, $p = 0.005$). Table 1 shows the relative frequency of the clinical manifestations of RF in children less than 7 years of age.

Laboratory findings

A complete blood count was also done for 60 patients, which showed leukocytosis with a WBC count more than 10,000 in 43% and a WBC count

Table 1. Comparison of clinical manifestations in different patient groups.

Clinical manifestation	All patients (%)	Children < 7 years (%)	Residents (%)	Visitors (%)
Fever	93.3	97.2	93.1	100
Chills	86.2	85.2	85.6	100
Headache	85.9	82.4	85.4	100
Nausea or vomiting	64.2	61.3	64.4	57
Sweating	64.1	58.5	63.8	71.4
Abdominal pain	57.4	57.7	58.8	42.9
Arthralgia	47.7	36.6	48.9	14.3
Cough	24.9	25.4	42.9	24.2
Photophobia	18.5	17.6	19.1	0
Epistaxis	9.2	11.3	9.6	0
Jaundice	4.4	0	4.5	0
Hematuria	3.8	2.1	4	0
Petichiae	2.8	3.5	2.9	0
Scleral congestion	2.6	2.1	2.6	7.1

less than 7000 in 20.7% of cases. The mean WBC count was 10,000 ± 3100 (neutrophils 74.9% ± 11.3%, Lymphocytes 23.1% ± 10.6% and eosinophils 0.8% ± 1.7%). Regardless of sex, hemoglobin level was less than 11mg/dL in 34% of cases and MCHC was 32.5 ± 1.8. Erythrocyte sedimentation rate was more than 40 mm in the first hour in 35 of 40 patients.

These findings were based solely on entomologic studies performed in the Khalkhal district, where 60 % of all cases of RF occurred.

Table 2. Relative frequency of soft ticks in the village colonies of Khalkhal

Village colony	Frequency (%)	F ± 2 SE
East Khandabil	12.8	12.8 ± 2 (0.19)
West Khandabil	15.7	15.7 ± 2 (0.19)
North Khorsh Rostam	9.4	9.4 ± 2 (0.16)
South Khorsh Rostam	10.1	10.1 ± 2 (0.2)
East Senjed	12.8	12.8 ± 2 (0.27)
Shal	9.1	9.1 ± 2 (0.2)
Palangah	5.4	5.4 ± 2 (0.3)
All regions	10.9	10.9 ± 2 (0.8)

Entomologic findings

Ornithodoros were found in 13 villages in Khalkhal. Among the 130 indoor sites studied, 1,421 ticks of the Argasidae family (soft ticks) were collected and identified. Of these, 45.9 %, 40.3 % and 13.8% were of *O. lahorensis*, *O. tholozani* and *Argas persicus*. Soft ticks were not found in 14 rodent nests.

Out of the 1,421 ticks collected, only 54 (48 *O. lahorensis* and six *O. tholozani*) were collected from human residences and the remainder were collected from animal shelters. *O. lahorensis* and *O. tholozani* were more prevalent in mountain areas whereas *A. persicus*, which is usually more common in the villages, was more common in non-mountainous areas. Soft ticks were more prevalent in indoor sites in West Khanehbil, with a prevalence of nearly 16 % (Table 2). The ticks collected from three villages in Khalkhal, namely Tazranaq (22 %), Khojin (12 %), and Biraq (9 %) were found to be infected with *Borrelia*.

Discussion

In this study, almost 90 % of the patients did not work outdoors and were comprised of children, housewives and students. It was not uncommon to keep cattle and sheep near or inside their homes. Our findings show that RF occurs more frequently

Table 3. Comparison of clinical manifestations of relapsing fever in four different studies.

Clinical manifestation	This study (%)	Brognolo[16] (%)	Brognolo (children)[15] (%)	Brown et al[17] (%)
Fever	93.3	99	100	73 (T > 39ºC)
Chills	86.2	—	74	30
Headache	85.9	92	84.5	88
Nausea or vomiting	64.1	—	—	46
Sweating	64.1	—	—	—
Abdominal pain	57.4	—	51	—
Arthralgia	47.7	51	—	—
Cough	24.9	—	—	—
Photophobia	18.5	—	—	—
Epistaxis	9.2	24	20	11
Jaundice	4.4	23	10	0
Hematuria	3.8	—	—	—
Petichiae	2.8	43	34	54
Scleral congestion	2.6	—	—	—

in indoor areas rather than outdoors, which correlates with the findings of Abidov et al, who studied RF in Uzbekistan.[6] As the *Otholozani* tick is rarely found in human residences, transmission of infection probably occurs through family contact. Most studies, including that of Vasil'eva, et al.[7] state that the most important risk factor involved in the transmission of infection is the close proximity of human residences and animal shelters.

Most cases of RF reported from endemic regions, were caused by an overnight stay in wooden shelters or inns in mountainous areas, or by traveling to endemic areas.[8,9] In our study, 16 patients who came from non-endemic areas (especially from Tehran) had become infected after traveling to endemic areas. As this figure only refers to those diagnosed with RF in the clinics or hospitals of Ardabil Province, we would expect the actual number of patients to be more. Hence, a patient with fever who has returned from an endemic region should be assumed to have RF.

Most cases of RF diagnosed in Ardabil Province were from Khalkhal, Kowsar and Nir districts. The prevalence was highest in Khandabil village (Table 2). However, the study performed by the Pasteur Institute of Iran in 1976 showed that RF was most prevalent in the village of Koraim (a county of Nir). In our study, although weak diagnostic tests were used, 38% of the patients who presented with fever finally developed RF.[10] RF had not been reported in the cities of Namin, Meshkin Shahr, Bileh Savar and Parsabad. Malaria and lyme disease are the most common afflictions to be considered in the differential diagnosis of RF. As stated in some articles, RF is rarely considered in the differential diagnosis of fever and chills in areas where malaria is the main health problem.[11,12] As malaria is highly prevalent in the cities of Parsabad and Bileh Savar, RF should be considered in the differential diagnosis of fever and chills in patients of these areas.

The majority of cases in our study were children, which is similar to the finding of Barclay and Coulter on tickborne RF in Tanzania.[12] However, the mean age of the infected children in their study was lower than ours: 88% of their patients were less than 5 years of age compared to 33% of the patients in our study.[1] Although differences between the population distribution pyramid of Iran and Tanzania may explain these findings, it does not fully explain the differences. In Barclay and Coulter's study, 36% of the cases were infants, whereas there were no infants in our study. Other differences may be due to numberous causes, including: the type of spirochete, genetic factors, entomologic factors, underdiagnosis of the infection in newborns, different methods of infant

care, and management of fever by healthcare personnel.[13] Some articles have reported vertical transmission as a possible route of acquiring the infection in newborns.[14,15] The most common clinical manifestations of RF in the present study were fever, chills and headache which is similar to other studies performed in this field. We found no studies on the symptoms of tickborne RF. The signs and symptoms of RF in our study can be compared with those of louseborne RF in the study by Borgnolo et al during the epidemic outbreak of RF in Ethiopia,[16,17] another study by Brongolo on 103 children hospitalized in an Italian hospital in Ethiopia,[16,17] and a study performed by Brown et al (Table 3).[18] Compared to the other studies, the incidence of petechiae in our study was very low (2.8% *vs.* 34%). This difference could be explained by the type of RF; for example, thrombocytopenia is a common finding in louseborne rather than in tickborne RF. Coughing, photophobia, sweating and hematuria were not indicated in the other studies. Considering the methodologic and statistical limitations of our study in identifying differences between the clinical manifestations of RF in those residing, or those visiting, the high prevalence area and the higher incidence of chills in females, the probable causes of these differences are not discussed here.

Leukocytosis is not an unexpected finding in RF and has been mentioned in many studies, including ours. Although anemia occurred in a large number of patients with RF, we are unable to provide possible explanations for this as there was no control group or randomization and, most importantly, anemia is common in Iran.

Although only 40 consecutive patients were assessed for ESR in our study, the mean values were higher than the normal range. With the exception of Dr. Karimi's report,[10] other studies and most textbooks do not mention high ESR values in RF. We suggest further research on tickborne RF based on laboratory findings. Due to the high prevalence of RF in Ardabil, further studies should be performed in this region.

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