Short communication

Rickettsia conorii specific Ig G antibodies: a seroepidemiologic survey in Constanta and Tulcea counties and Bucharest, Romania, 2009

Anticorpi specifici Ig G *Rickettsia conorii*: studiu seropidemiologic în județele Tulcea şi Constanța şi în Bucureşti, România, în 2009

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Abstract

Introduction. Boutonneuse fever (BF) is an emerging infectious disease in Romania evolving in the southern part of the country, as revealed by the incidence rates registered in our previous epidemiological descriptive studies performed during last years. We aimed to evaluate the presence of Rickettsia conorii specific IgG antibodies in a population sample from the affected area. Methods. An ELISA serological survey was performed on 301 serum samples collected from the general population, living in an area most affected by BF, in southern Romania. One of the selection criteria was the absence of signs and symptoms of the disease during the seasonal development of BF. Data were processed in SPSS V.19 (indicators of central tendency, dispersion, frequency and benchmarking). Results. Serological results showed a general positivity rate of 25.2%, with the highest rate in the population group aged over 60 years, this age group accounting for 34.21 % of the whole sample (55.6% for Bucharest area and 63.16% for Tulcea district). Conclusions. By confirming the hypothesis based on our previous descriptive epidemiological studies that BF is fitting an endemic pattern in southern Romania, our study results strongly support the need to set up a national surveillance program, aiming at improving BF prevention, management and control in our country. To our knowledge, this is the first study on Rickettsia conorii specific IgG antibodies seroprevalence in Romania.

Keywords: *Boutonneuse Fever, seroprevalence, southern Romania*

Rezumat

Introducere. Febra butonoasă (FB) este o boală infecțioasă emergentă în România, evoluând în sudul țării, după cum reiese din rata incidenței înregistrată în ultimii ani, conform studiilor noastre anterioare de epi-

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demiologie descriptivă. Ne-am propus să evaluăm prevalenţa anticorpilor specifici IgG Rickettsia conorii într-un eşantion de populaţie generală din aria afectată. Metode. S-a efectuat un studiu de seroprevalenţă prin metoda ELISA pe 301 probe de ser colectate din aria de sud a României; aria cea mai afectată de FB. Unul din criteriile de selecţie a fost absenţa semnelor şi simptomelor caracteristice bolii în perioada de evoluţie sezonieră a FB. Analiza statistică. Datele au fost prelucrate statistic cu softul SPSS V.19 (indicatori tendinta centrala, dispersie, frecventa si teste de comparare). Rezultate. Rezultatele serologice au arătat o rata de pozitivitate generală de 25.2% cu cea mai mare rată de pozitivitate la grupul de populaţie de peste 60 de ani 34,21% din totalul probelor de ser (63,6% pentru judeţul Tulcea şi 55.56% pentru Bucureşti). Concluzii. Confirmând ipoteza că FB îmbracă un caracter endemic în sudul României, bazată pe studiile noastre anterioare, studiul de faţă a adus argumente în sprijinul necesităţii de a introduce un program naţional de supraveghere în scopul cunoaşterii reale a incidenţei, managementului şi controlului FB în ţara noastră. După cunoştintele noastre, acesta este primul studiu de seroprevalenţă a anticorpilor specifici IgG Rickettsia conorii în România.

Introduction

Since mankind has existed, fleas and lice have been its permanent companions. The price of this companionship is represented by the resulting communicable diseases. Recent studies have shown that the presence of *Rickettsia prowazekii* nucleic acids was confirmed in soldiers of Napoleon's French army during his Russian campaign (1).

Boutonneuse fever (BF) is a tick-borne disease caused by *Rickettsia conorii*. It was first described a century ago as a disease with high fever and spots (2).

Rickettsia conorii belongs to the spotted fever group of the genus *Rickettsia* (family *Rickettsiaceae*, order *Rickettsiales*) and is an obligate intracellular coccobacillus that grows within the cytoplasm of its eukaryotic host cells (3).

Boutonneuse fever is often severe and even a fatal zoonozis, transmitted in Europe mainly by *Rhipicephalus sanguineus*, known as "the brown dogtick".

In Romania, boutonneuse fever was detected for the first time in 1910 by Serafidi. In 1931 V. Vasiliu, G. Barzanescu and D. Combiescu described the characteristics of the illness (4-6).

In a previous descriptive epidemiological study performed in Romania, 2000 – 2008, all cases of BF recorded nationwide came from Southern Romania. The highest rate of incidence, $1.6\%_{000}$, occurred in 2002 and represented 22.9% of all cases reported in that period. The high rate of incidence reported in counties Constanta, Tulcea and Bucharest area suggested that BF could be a public health problem for these areas (7).

The temporal distribution respected the maximum activity interval of the vector *R. sanguineus*. The historical seasonality for the boutonneuse fever is June-September; in our previous study we found a trend of extending the reporting interval, as the cases were detected beginning with May. The evolution of boutonneuse fever cases matched the sporadic case pattern (7).

By our present study we aimed to evaluate the prevalence of *Rickettsia conorii* specific IgG antibodies in a general population sample from the affected area, in order to appreciate the opportunity to set up a national surveillance program of BF in Romania.

Material and methods

We performed a serological survey in 2009, on 301 serum samples collected from a randomized sample of the general population selected from areas most affected by BF in southern Romania: Constanta and Tulcea counties and Bucharest.

Table 1. Distribution of the serological results by county

ELISA Results	County							
	Total		Bucharest		Constanta		Tulcea	
	Count	%	Count	%	Count	%	Count	%
Total	301	100%	99	32.9%	119	39.5%	83	27.6%
Equivocal	14	4.7%	12	4.0%	2	0.7%	0	0.0%
Negative	211	70.1%	69	22.9%	78	25.9%	64	21.3%
Positive	76	25.2%	18	6.0%	39	13.0%	19	6.3%

According to the incidence data from the year 2008 ($11.11\%_{000}$ rate of incidence for Constanta county $15.26\%_{000}$ rate of incidence for Tulcea county and $0.41\%_{000}$ for Bucharest area) (7), the representative population sample should have been 360 persons. After applying our selection criteria (volunteers without signs or symptoms of the disease investigated for other diseases in the period of time that corresponded to the maximum activity of the *R. sanguineus* tick according to the lab capacity), our sample included 301 persons.

All persons included in this serologic study, or their parents for children younger than 18 years, signed an informed consent form expressing their agreement to participate in this study. Sex and age were recorded for all persons included.

Sera were stored at –20°C until tested at the National Institute of Public Health, Bucharest, for IgG *R. conorii* specific antibodies, by an ELISA method, using an ImmunoLISA *Rickettsia conorii* IgG/IgM kit (Orgenics Ltd). Performance parameters of the ELISA kit used in this study were: 100% specificity and 85% sensitivity, as determined by the manufacturer.

Antibody index was defined as the fraction between sample O.D. (optical density) and cut off serum mean O.D. multiplied by 10. The antibody index for a positive reaction was >11, as indicated by the manufacturer. Equivocal results were in the range of 9-11, while antibody indexes less than 9 were considered negative reactions.

Samples with indexes below 9 were considered as not having IgG specific antibod-

ies against *R. conorii*. Samples with indexes above 11 were considered as having IgG specific antibodies against *R. conorii*. Samples with equivocal results were retested.

Data were processed with SPSS V 19. The distribution by age of our sample was tested using statistics of central tendency, histogram of ages and measurements of the central tendency. Differences in the distribution of the positive samples among the age groups according to the county of provenience were tested using Kruskal-Wallis test, significant difference was considered when $p<0.05$.

Results

In this study were enrolled 301 persons.

The highest positive rate was found in Constanta county (51.32 % from the total positive samples). The distribution of the serological results is presented by county in *Table 1*.

From the tested sera, 161 (53.49%) samples were collected from females. The distribution of positivity by gender and county is presented in *Table 2*.

Statistical analysis of the age of the selected population sample displayed an asymmetrical distribution curve showing right skewness with the larger number of subjects having ages over 21 years (79 cases)

The amplitude of age group distribution was 90 years (inferior limit 1 year and superior limit 90 years), suggesting that the disease

Table 2. Distribution of positive samples by gender and county

County	Female	Male	Total
Bucharest	11 (29.7%)	7 (17.9%)	18 (23.7%)
Constanta	17 (45,9%)	22 (56.4%)	39 (51.3%)
Tulcea	9 (24.3%)	10 (25.6%)	19 (25%)
Total	37 (100%)	39 (100%)	76 (100%)

Table 3. Seropositivity rates by age group and geographical area

County	1-20 y	21-30 y	31-40 y	41-50 y	51-60 y	Over 60 y	Total
Bucharest	2 (11.11)	5 (27.78)	0	0	1 (5.56)	10 (55.56)	18
Constanta	5 (12.82)	8 (20.51)	5 (12.82)	8 (20.51)	9 (23.08)	4 (10.26)	39
Tulcea	0	2 (10.53)	1 (5.26)	0	4 (21.05)	12 (63.16)	19
Total	7 (9.21)	15 (19.74)	6 (7.89)	8 (10.53)	14 (18.42)	26 (34.21)	76 (25.20)

Results expressed as number of positive samples (percentage).

reaches all groups of age. The group was strongly inhomogeneous for age distribution (standard deviation is 19.5 years) with a median age of 39 years and a modal age of 29 years.

Of the tested sera, 25.20% were positive for the presence of Ig G *R. conorii* specific antibodies. Most *R. conorii* specific IgG positive sera were found in the 60+ age group, followed by the 21 - 30 age groups (*Table 3*). The highest positive rate was registered in Constanta county (13.%).

From the 76 positive sera, 53.16 % were from females, while 46.84% were from males: the positive rate by gender was 23.12 % for females and 27.65% s for males.

The highest positive rate was found in the age group of over 60 years, both in the general sample (34.21%) and for each of the studied geographical areas: 66.67%, 47.61% and 35.29% for Constanta county, Bucharest and Tulcea county, respectively. For Constanta county we found 5 cases out of 13 positive for *Rickettsia conorii* specific IgG antibodies, belonging to the 0 – 21 age group. This finding has to be further investigated, as it represents clinically and epidemiologically valuable information which needs to be integrated (*Table 3*).

Because the age distribution of our sample was inhomogeneous we tested the hypothesis according to which there were statistically significant differences between the median ages of the positive cases between counties, using the Kruskal-Wallis test. This hypothesis was rejected (p=0.0001).

Discussions

Transmission of rickettsiae in humans may occur directly, as a result of infected tick bite or crushing the tick on the skin, or indirectly, by mucosal entry, through contaminated hands (8).

BF is known also as Mediterranean spotted fever (MSF) or Israeli spotted fever.

The abundance of the tick itself is an important determinant of the disease prevalence and is influenced by many factors, including climatic and ecologic conditions (9, 10). Climatic variations are suspected to play an important role in tick activity and, consequently, on rickettsial prevalence (11,12).

The risk of ticks transmitting *Rickettsiae* and the prevalence of a specific tickborne disease is dependent on several parameters. The

prevalence of *Rickettsia* - infected ticks, which can vary greatly, is important. For example, up to 12% of *Rhipicephalus sanguineus* ticks are infected with *R. conorii* in southern France (13), whereas only 0.5% of *D. variabilis* ticks in North Carolina are infected by *R. rickettsii* (14). The affinity of a specific tick for human beings also varies. For example, in Mediterranean countries, although nearly everybody is in contact with the dog tick *Rhipicephalus sanguineus*, the prevalence of BF is only 50 per 100,000 inhabitants. The reason is the low affinity of this tick for hosts other than the dog.

A study performed in Israel demonstrated that *Rhipicephalus sanguineus* ticks fed on dogs and hedgehogs were infected with *R. conorii* but the role of vertebrate reservoirs in maintaining zoonotic foci has yet to be clarified (15).

Recently BF appears to be a more severe disease than it was in the past. Thus, reported mortality rates were 3.2% in Oran, Algeria, in 2004 (16) and even 32.3% in hospitalized patients in Beja, Portugal, in 1997 (17). This increase in severity has not been explained yet. But BF is now more quickly recognized and treated with more effective antimicrobial drugs than in the past. Based on these, we are expecting better outcome of BF. This was the case for RMSF (Rocky Mountain spotted fever), which caused mortality rates in the United States of 2.4% during 1993–1996 (18) and 1.4% during 1997–2002 (19), in contrast to a mortality rate of 65%–90% at the beginning of the century.

A hypothesis explaining the higher mortality rates could be that severe forms of spotted fever cases were not easily recognized in the near past. In fact, BF was considered to be a benign disease before the 1980s (20). BF was not considered as a possible diagnosis for patients hospitalized in intensive care units with a febrile rash. Compared to RMSF, BF was considered a benign disease for 70 years, therefore its real severity was long time ignored. Although the mortality rate was evaluated to be 1-3% in the early reports in the literature, the first description of a highly severe form of BF was published in the early 1980s (21). Nowadays, we know that BF is at least as severe as RMSF.

Another hypothesis for the higher mortality could be that more virulent strains of *R. conorii* appeared in last years (22).

Data from the Romanian National Centre for Statistics compared national and regional incidence of BF between 2000 and 2009. According to these, BF may represent a public health problem for some regions. Tulcea, Constanta and Bucharest areas presented high prevalence of BF cases over the years: in 2001 the highest prevalence was registered in Constanta (44.2%$_{000}$), in 2002 the prevalence of BF was 39%$_{000}$ in Tulcea; for Bucharest the prevalence over the years was higher than in the rest of the southern Romanian districts (for example, in 2003 it was 2 times higher than the national baseline level) (23).

Reported cases from our previous epidemiological study had serological diagnosis performed in only 38% (7). Data obtained in the present serological study showing a positive rate of 25.2% of the sample (13% Constanta, 6% Bucharest and 6.3% Tulcea) strengthen the idea that BF is a public health problem in these areas.

Possible bias in respect of positive rates by geographical area may be due to the fact that our sample is not a representative one, this highlighting again the need for BF surveillance.

Conclusions

Although our general population sample was not representative for the counties, by the present seroprevalence study we reached our goal to better understand BF seroepidemiology in Romania. It strengthens the hypothesis based on our previous descriptive epidemiological studies that this tick borne zoonosis is fitting an endemic pattern in southern Romania.

Evidence provided strong support for the necessity to further investigate the real amplitude of the disease and promotes the need to

set up a national surveillance program, aiming the prevention, management and therapy of BF in our country.

To our knowledge, this is the first sero-epidemiological study of BF in Romania.

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