ORIGINAL ARTICLE 10.1111/j.1198-743X.2004.00782.x

Dermacentor-borne necrosis erythema and lymphadenopathy: clinical and epidemiological features of a new tick-borne disease

J. A. Oteo[1], V. Ibarra[1], J. R. Blanco[1], V. Martínez de Artola[2], F. J. Márquez[3], A. Portillo[1], D. Raoult[4] and P. Anda[5]

[1]Servicio de Medicina Interna y Enfermedades Infecciosas, Hospital de La Rioja, Logroño, [2]Sección de Microbiología, Hospital Virgen del Camino, Pamplona, [3]Departamento de Biología Animal, Universidad de Jaén, Spain, [4]Unité des Rickettsies, Université de la Méditerranée, Marseille, France and [5]Servicio de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

ABSTRACT

This paper describes the epidemiological and clinical features of a tick-borne disease differing somewhat from other tick-borne diseases found previously in Spain. All patients were bitten by *Dermacentor marginatus* or a large tick. The clinical features include a crustaceous or necrotic lesion at the site of the tick's attachment, surrounded by an erythema (erythema migrans-like) and painful regional lymphadenopathies. The probable aetiological agent is *Rickettsia slovaca*. Similar cases have been reported in other European countries.

Keywords *Dermacentor* spp., *Rickettsia*, tick-borne disease, zoonoses

Original Submission: 21 May 2002; **Revised Submission:** 29 March 2003; **Accepted:** 24 April 2003

Clin Microbiol Infect 2004; 10: 327–331

INTRODUCTION

Hard ticks are haematophagous arthropods that feed on different mammals, birds and reptiles. They can transmit a broad variety of infectious and toxic diseases, but humans are only occasional hosts. Tick-borne diseases (TBDs) result from at least three elements: the geographical presence of each tick species, the presence of a transmissible agent, and a susceptible host [1,2]. This paper describes 22 patients with a TBD not described previously in Spain. Raoult *et al.* [3] and Lakos [4] have described cases with similar characteristics in other countries, and have implicated *Rickettsia slovaca* as the aetiological agent. The vector is *Dermacentor marginatus*. A preliminary description of this new TBD in Spain has been published elsewhere [5].

PATIENTS AND METHODS

Study site

All patients were living in La Rioja, a region of Northern Spain, and were diagnosed in Hospital de La Rioja, Lograño, which is the regional reference centre for zoonoses (including TBD) and serves an area with >260 000 inhabitants. The region has different ecological habitats, some of which (e.g., mountains) are favourable for Lyme borreliosis and human granulocytic ehrlichiosis (HGE) [6], while others (e.g., valleys) favour Mediterranean spotted fever [7]. The study was prospective (January 1999 to April 2001) for seven patients and retrospective (January 1990 to December 1998) for 15 patients.

Corresponding author and reprint requests: J. A. Oteo, Hospital de La Rioja, Avd Viana 1, ES-26001 Logroño (La Rioja), Spain
E-mail: medoteo@saludalia.com

Patients

Criteria for inclusion

1. Patients bitten by *Dermacentor* spp. or a large tick during the period of maximum activity for *D. marginatus* (in our area, October to April) were included. Patients bitten by *Ixodes ricinus*, or *Rhipicephalus sanguineus*, or those who had been bitten in a period of maximum activity of these ticks (from the beginning of May to the end of September) were excluded.
2. All included patients had a crustaceous eruption or a point of necrosis (eschar) at the tick's attachment site, surrounded by erythematous skin and regional lymphadenopathy.
3. Included patients had negative serological tests (acute and convalescent sera) for *Borrelia burgdorferi* (*B. burgdorferi* Virotech ELISA IgG and IgM; Genzyme, Neu-Isenberg, Germany), *Francisella tularensis* (*F. tularensis* antigen tube test; Difco, Detroit, MI, USA), and HGE (HGE immunofluorescence assay for IgG; MRL Diagnostics, Cincinatti, OH, USA). The presence of antibodies against *Rickettsia conorii* (*R. conorii* IFI assay; BioMérieux, Lyon, France) was not considered to be a reason for exclusion, because there is a serological cross-reaction between the two *Rickettsia* spp. [8].

The following additional microbiological studies (not necessary for inclusion) were performed for some patients, all of whom matched the above criteria.

Determination of antibodies against R. slovaca
Immunofluorescent assays were performed in the Unité des Rickettsies, Marseille, France, and in the Centre of Estudos de Vectores e Doenas Infecciosas, Aguas de Moura, Portugal.

Skin biopsy
In three patients, a skin biopsy (punch and/or conventional biopsy) was performed to investigate the presence of spotted fever group rickettsias by PCR (amplification with citrate synthase primers) as described by Roux *et al.* [9]. The possible presence of *B. burgdorferi* was also investigated by means of culture (BSK medium) and PCR, as described previously [10].

Tick study
A specimen of *D. marginatus*, collected from a patient with the typical clinical picture in 1996, was investigated by PCR (citrate synthase gene) for *Rickettsia* spp. [9]. The possible presence of *B. burgdorferi* was also investigated by means of culture (BSK medium) and PCR, as described previously [10].

RESULTS

Epidemiological data

Twenty-two (13.2%) of 167 patients with TBD diagnosed during the study were included. The main epidemiological and clinical characteristics of the patients are shown in Table 1. The average age was 37.5 years (range: 3–67 years), with 45.5% male and 54.5% female patients. The monthly distribution of the tick bites is shown in Fig. 1. All tick bites occurred during the colder months, with a peak in November. In 11 (50%) cases, *D. marginatus* was identified or recognised by the patients from a tick image collection. In the remaining cases, a large engorging tick bite was reported.

Clinical data

The main signs and symptoms present in all the patients were a crustaceous lesion (early) or eschar (late) at the site of the tick's attachment (Fig. 2) and regional painful lymph nodes. Surrounding erythema was present in all patients, and this was ≥5 cm in diameter (erythema migrans-like) in 36.6% of patients (Fig. 3). One patient had an erythema that was >40 cm in diameter. All eschars were located on the upper portion of the body, usually on the occipital scalp (86.4%). Low-grade fever (< 38 °C) was present in 45.5% of patients. All but one patient with eschar in the scalp suffered from headache. The mean incubation period after the tick bite was 4.2 days (range: 1–8 days). No other skin lesions were detected.

Routine laboratory tests were normal in 17 patients, but five (22.7%) had raised alanine aminotransferase and aspartate aminotransferase levels (less than twice the upper limit of normal levels). All patients, with the exception of a child who received josamycin, were treated with oral

Table 1. Epidemiological and clinical characteristics of the patients

Case	Age	Sex	Month	Village	Location of eschar	Tick	Incubation (days)	Erythema	Regional lymph nodes	Fever	Headache	Transaminases
1	60	F	April	Viguera	Head	UN	5	Yes[a]	Yes	Yes	Yes	Elevated
2	51	M	January	Arnedo	Head	DM	7	Yes	Yes	Yes	Yes	Elevated
3	59	F	November	Arnedo	Arm	DM	6	Yes[a]	Yes	No	No	Normal range
4	26	F	March	Arnedo	Head	DM	4	Yes	Yes	No	No	Normal range
5	11	F	November	UN	Head	DM	4	Yes	Yes	No	Yes	Normal range
6	28	F	December	UN	Head	UN	7	Yes	Yes	Yes	Yes	Normal range
7	44	F	February	UN	Head	UN	5	Yes	Yes	No	Yes	Normal range
8	7	M	October	UN	Head	UN	5	Yes	Yes	Yes	Yes	Normal range
9	3	F	October	UN	Head	DM	3	Yes	Yes	No	Yes	Normal range
10	39	F	November	Logroño	Head	UN	4	Yes	Yes	Yes	Yes	Normal range
11	29	M	November	Sotes	Head	DM	3	Yes	Yes	Yes	Yes	Elevated
12	31	F	December	Préjano	Head	DM	4	Yes	Yes	Yes	Yes	Normal range
13	44	F	January	M. de Ocón	Head	UN	3	Yes[a]	Yes	No	Yes	Normal range
14	66	F	October	Arnedo	Head	DM	6	Yes	Yes	No	Yes	Normal range
15	21	M	December	Arnedillo	Head	UN	4	Yes	Yes	Yes	Yes	Normal range
16	67	M	November	Arnedo	Chest	DM	8	Yes[a]	Yes	No	No	Normal range
17	49	F	March	Arnedo	Head	UN	5	Yes[a]	Yes	Yes	Yes	Elevated
18	22	M	March	Aldeanueva	Head	DM	4	Yes[a]	Yes	No	Yes	Normal range
19	45	M	April	Tudelilla	Axilla	DM	8	Yes[a]	Yes	No	No	Normal range
20	49	M	April	V. de Ocón	Head	UN	1	Yes	Yes	Yes	Yes	Normal range
21	25	M	January	UN	Head	UN	3	Yes	Yes	No	Yes	Normal range
22	48	M	January	Sta. Engracia	Head	UN	1	Yes[a]	Yes	No	Yes	Elevated

DM, *Dermacentor marginatus*; F, Female; M, Male; NP, not performed; UN, unknown.
[a]Erythema >5 cm in diameter.

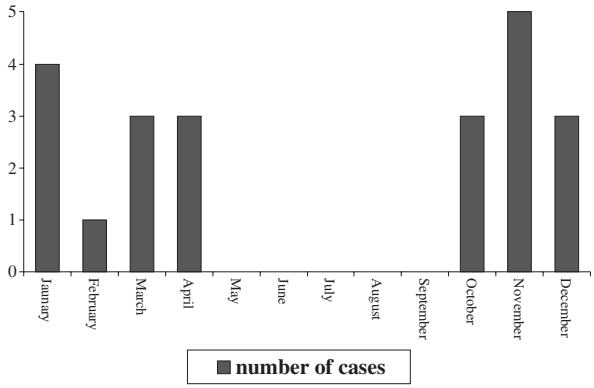

Fig. 1. Distribution of cases by months.

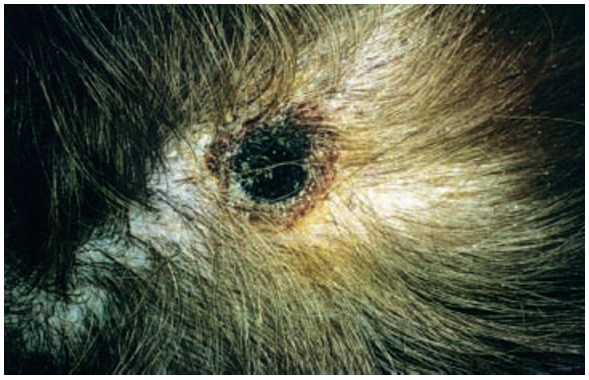

Fig. 2. Detail of the eschar on the scalp.

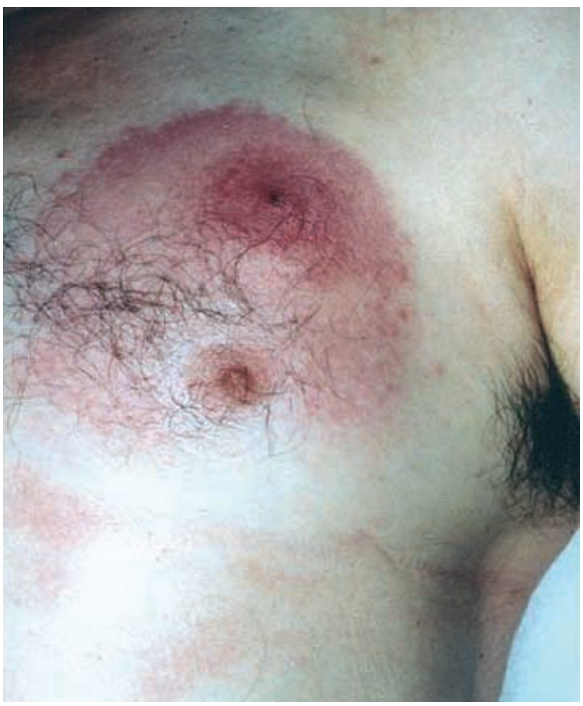

Fig. 3. Eschar (*tache-noire*-like) at the point of the tick's attachment, surrounded by erythema migrans-like lesion.

doxycycline (100 mg twice-daily for 14 days). The resolution of lymphadenopathies in a few days was the rule, but the eschar had a torpid evolution that resulted in alopecia lasting for several months. None of the patients were hospitalised, and all had a favourable resolution.

Serological and microbiological findings

Serological assays for *R. slovaca* were performed for 12 (55.5%) patients; seven of these were prospective, and stored sera were available for five of them. A low titre, with a previous negative result, was observed for only three patients. These three patients also had a serological response against *R. conorii*. Antibody titres against *R. slovaca and R. conorii* were similar (Table 2).

The results of all microbiological investigations (culture in BSK medium, PCR for *B. burgdorferi*, and PCR for *Rickettsia* spp.) were negative in the three patients where skin biopsies were analysed.

For case 16 (Table 2), it was possible to investigate the tick (an adult engorged female *D. marginatus*) by PCR. The results were positive for *Rickettsia* spp., and subsequent sequencing indicated that the species involved was *R. slovaca* (unpublished results).

DISCUSSION

Different TBDs have now been described, both in Europe and worldwide [1,11]. Lyme borreliosis and Mediterranean spotted fever are the most prevalent TBDs in Spain [2,12], but other TBDs, such as HGE [13], tick paralysis [14], babesiosis and tularemia [15] have been described.

This paper presents data about a new TBD in Spain. The vector is *D. marginatus*, and the clinical picture is different from the other TBDs described in the area. A point of necrosis (eschar) at the site of the tick's attachment, surrounded by an erythematous skin lesion and regional lymphadenopathy, are the most prominent signs of this disease. The epidemiological data are also different from those of the other TBDs, since the tick bite occurs during the colder months of the year.

Although there are clinical and epidemiological differences compared to the other TBDs described in Spain, there is a great similarity to the patients described in France and Hungary by Raoult *et al.* [3] and Lakos [4], respectively. The disease appears during the cold months, unlike Lyme

Table 2. Results of the serological and PCR assays

	R. conorii		*R. slovaca*			PCR from the skin	
Case	**IgM**	**IgG**	**IgM**	**IgG**	**Skin biopsy**	***B. burgdorferi***	***R. slovaca***
1	Negative	Negative	NP	NP	NP	NP	NP
2	IgM 1/32	Negative	NP	NP	NP	NP	NP
3	Negative	Negative	NP	NP	NP	NP	NP
4	Negative	Negative	NP	NP	NP	NP	NP
5	Negative	Negative	Negative	Negative	NP	NP	NP
6	IgM 1/64	Negative	NP	NP	NP	NP	NP
7	Negative	Negative	NP	NP	NP	NP	NP
8	IgM 1/16	IgG 1/32	IgM 1/16	IgG 1/32	NP	NP	NP
9	Negative	Negative	NP	NP	NP	NP	NP
10	IgM 1/128	IgG 1/128	NP	NP	NP	NP	NP
11	IgM 1/16	IgG 1/128	IgM 1/16	IgG 1/128	NP	NP	NP
12	IgM 1/32	IgG 1/128	NP	NP	NP	NP	NP
13	IgM 1/64	IgG 1/128	NP	NP	NP	NP	NP
14	Negative	IgG 1/32	Negative	Negative	NP	NP	NP
15	IgM 1/16	IgG 1/64	IgM 1/16	IgG 1/64	NP	NP	NP
16[a]	Negative	Negative	Negative	Negative	Performed	Negative	Negative
17	Negative	Negative	Negative	Negative	NP	NP	NP
18	Negative	Negative	Negative	Negative	NP	NP	NP
19	Negative	Negative	Negative	Negative	Performed	Negative	Negative
20	Negative	Negative	Negative	Negative	Performed	Negative	Negative
21	Negative	Negative	Negative	Negative	NP	NP	NP
22	Negative	IgG 1/128	Negative	Negative	NP	NP	NP

NP, not performed.
[a]For case 16, PCR from the tick was positive for *Rickettsia* spp.

borreliosis, Mediterranean spotted fever or ehrlichiosis. The clinical picture partly resembles Lyme borreliosis (erythema migrans-like lesion) and Mediterranean spotted fever (*tache noire*-like lesion at the tick's attachment site), but the site of the tick bite differs, being located mostly on the scalp (86.4% of patients) and always on the upper portions of the body. As with early localised Lyme borreliosis, but in contrast to spotted fever, the systemic signs and symptoms are not prominent. The only symptoms are headache (95% when the tick attachment is on the scalp) and the development of an enlarged painful regional lymphadenopathy. Only 45.5% of patients had low-grade fever, and even fewer cases (22.7%) had elevated liver enzymes.

The disease vector, *D. marginatus*, is a hard tick (Ixodidae), present in all parts of Europe, that feeds on a variety of different mammals. In Spain, it feeds mainly on wild boars (*Sus scrofa*) during its adult stage. Our microbiological data are not sufficient to definitively confirm *R. slovaca* as the aetiological agent of this TBD. A poor and late serological response against this organism was observed in 25% of patients analysed. Similar findings were reported by Raoult *et al.* [16]. All patients with antibodies against *R. slovaca* also showed low titres of antibodies against *R. conorii* because of cross-reaction (both of these organisms are included in the same group of rickettsiae) with immunofluorescence assays [8]. However, the assay for *R. conorii* is currently the best serological marker that is widely available for this infection, since 45.5% of patients had antibodies against *R. conorii* (an assay for *R. slovaca* is currently only available in specialist reference laboratories). *R. slovaca* was found in an engorged female *D. marginatus* tick attached to a patient with the typical clinical picture. *R. slovaca* was also detected in the border of the lesion (skin biopsy) and in one tick after PCR and sequencing in the first case report described by Raoult *et al.* [3]. Lakos [17] found the same species by PCR in Hungarian patients. Overall, the data presented in the present study indicate that clinicians should be aware that this tick-related disorder can be found in Spain as well as in other parts of Europe.

ACKNOWLEDGMENTS

We thank Dr Fatima Bacellar (CEVDI, Portugal) for her contribution and collaboration in the performance of serological assays. This study was supported in part by a grant from the Fondo de Investigación Sanitaria (FIS-PI021810) from the Ministerio de Sanidad y Consumo, Spain.

ADDENDUM

Since the preparation of this paper, we have analysed DNA from six *D. marginatus* ticks collected from patients and 38 from wild boars by a semi-nested PCR (*ompA* gene) with the primers described by Regnery *et al.* [18]. We have also analysed a blood sample from a patient following the same protocol. All tick samples of clinical origin, and 33 of 38 from mammals, showed

the presence of the *ompA* gene of *Rickettsia* spp. Five samples (four from patients and one from a wild boar) were sequenced. In two cases (one from a wild boar and one from a tick collected from a patient), the amplicons were identical to the *ompA* gene belonging to *R. slovaca*. In the remaining cases (one from blood and three from ticks), the amplicons showed highest similarity (98–97%) to *Rickettsia* sp. JL-02, *Rickettsia* sp. RpA4, *Rickettsia* sp. DnS14 and *Rickettsia* sp. DnS28.

REFERENCES

1. Parola P, Raoult D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin Infect Dis* 2001; **32**: 897–928.
2. Oteo JA. Ticks. A century of tick-borne diseases (*Ixodoidea*). *Rev Clin Esp* 1995; **195**: 1–2.
3. Raoult D, Berbis P, Roux V, Xu W, Maurin M. A new tick-transmitted disease due to *Rickettsia slovaca*. *Lancet* 1997; **350**: 112–113.
4. Lakos A. Tick-borne lymphadenopathy—a new rickettsial disease? *Lancet* 1997; **350**: 1006.
5. Oteo JA, Ibarra V. DEBONEL (Dermacentor-borne-necrosis-erythema-lymphadenopathy). A new tick-borne disease? *Enferm Infecc Microbiol Clin* 2002; **20**: 51–52.
6. Estrada-Peña A, Oteo JA, Estrada-Peña R *et al*. *Borrelia burgdorferi* in ticks (*Acari: Ixodidae*) from two different foci in Spain. *Exp Appl Acarol* 1995; **19**: 173–180.
7. Oteo JA, Estrada-Peña A, Ortega C, Martínez de Artola V. Mediterranean spotted fever. A preliminary tick field study. *Eur J Epidemiol* 1996; **12**: 475–478.
8. La Scola B, Raoult D. Laboratory diagnosis of rickettsiosis: current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997; **35**: 2715–2727.
9. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for rickettsiae. *Int J Syst Bacteriol* 1997; **47**: 252–261.
10. Oteo JA, Backenson PB, Vitutia MM *et al*. Use of the C3H/He Lyme disease model for the recovery of *Borrelia garinii* from erythema migrans lesions. *Res Microbiol* 1998; **149**: 39–46.
11. Parola P, Raoult D. Tick-borne bacterial diseases emerging in Europe. *Clin Microbiol Infect* 2001; **7**: 80–83.
12. Oteo JA, Blanco JR, Martínez de Artola V, Grandival R, Ibarra V, Dopereiro R. Migratory erythema (Lyme borreliosis). Clinicoepidemiologic features of 50 patients. *Rev Clin Esp* 2000; **2**: 60–63.
13. Oteo JA, Blanco JR, Martínez de Artola V, Ibarra V. First report of human granulocytic ehrlichiosis from southern Europe (Spain). *Emerg Infect Dis* 2000; **6**: 430–432.
14. Oteo JA, Maraví E, Martínez de Artola V, Antuñano P. Tick paralysis. *Med Clin (Barc)* 1990; **94**: 275–276.
15. Oteo JA. Tick-borne diseases in Spain [abstract S229]. In: *Abstracts of the 11th European Congress of Clinical Microbiology and Infectious Diseases, Istanbul, Turkey*: European Society for Clinical Microbiology and Infectious Diseases, 2001; 31.
16. Raoult D, Lakos A, Fenollar F, Beytout J, Brouqui P, Fournier PE. Spotless rickettsiosis caused by *Rickettsia slovaca* and associated with *Dermacentor* ticks. *Clin Infect Dis* 2002; **34**: 1331–1336.
17. Lakos A. Tick-borne lymphadenopathy (TIBOLA) [abstract S228]. In: *Abstracts of the 11th European Congress of Clinical Microbiology and Infectious Diseases, Istanbul, Turkey*: European Society for Clinical Microbiology and Infectious Diseases, 2001; 31.
18. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of Rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* 1991; **173**: 1576–1589.