Epidemiol. Infect. (2005), **133**, 943–949. © 2005 Cambridge University Press
doi:10.1017/S0950268805004061 Printed in the United Kingdom

Tick species and tick-borne infections identified in population from a rural area of Spain

FRANCISCO JESÚS MERINO[1], TERESA NEBREDA[1]*, JOSE LUIS SERRANO[2], PEDRO FERNÁNDEZ-SOTO[3], ANTONIO ENCINAS[3] AND RICARDO PÉREZ-SÁNCHEZ[4]

[1] *Servicio de Microbiología, Hospital Santa Bárbara, Complejo Hospitalario de Soria, Soria, Spain*
[2] *Servicio Territorial de Sanidad y Bienestar Social, Soria, Spain*
[3] *Departamento de Parasitología, Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain*
[4] *Departamento de Patología Animal, IRNA-CSIC, Salamanca, Spain*

(*Accepted 15 January 2005*)

SUMMARY

To determine the tick species that bite humans in the province of Soria (Spain) and ascertain the tick-borne pathogens that threaten people's health in that province, 185 tick specimens were collected from 179 patients who sought medical advice at health-care centres. The ticks were identified, and their DNA examined by PCR for pathogens. Most ticks were collected in autumn and spring (59 and 57 respectively). Nine species of ticks were identified, the most frequent being *Dermacentor marginatus* (55·7%), *Ixodes ricinus* (12·4%) and *Rhipicephalus bursa* (11·9%). Ninety-seven females, 66 males, 21 nymphs and one larva were identified. Twenty-six ticks carried DNA from *Rickettsia* spp. (11 *Rickettsia slovaca*, 6 *Rickettsia* spp. RpA4/DnS14, 1 *Rickettsia massiliae*/Bar29, and 8 unidentified); two ticks carried DNA from *Borrelia burgdorferi sensu lato* and seven ticks harboured DNA from *Anaplasma phagocytophilum*.

INTRODUCTION

Ticks are obligate blood-sucking arthropods that parasitize vertebrates. They are distributed throughout the world, mainly in rural areas [1]. There are over 800 known tick species but just a few of them, belonging to the genera *Ixodes*, *Rhipicephalus*, *Amblyomma*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Argas* and *Ornithodoros*, feed on, and thus can transmit pathogens to, human beings [1, 2]. Ticks transmit a broad range of infectious agents (viruses, bacteria, parasites) and, after mosquitoes, are the most important vectors of diseases for humans [1, 3, 4]. In addition to their role as vectors, ticks can also act as reservoirs for some of the pathogens that they harbour [1].

Environmental conditions such as climate, vegetation, and abundance of hosts, limit the geographic distribution of the ticks and the pathogens they transmit. This means that many tick-borne pathogens are restricted to particular areas: for example *Rickettsia rickettsii* in North and South America; *R. sibirica* in the northeast of central Asia and China; *R. conorii* in the south of Europe, Southeast Asia, India and Africa, and *Borrelia burgdorferi sensu stricto* (*s.s.*) in Europe and North America. However, the distribution of the tick-borne pathogens may change if that of their vectors and reservoirs does, perhaps through climatic changes, deforestation and/or migrations of humans and animals [1].

* Author for correspondence: Dr T. Nebreda, Servicio de Microbiología, Hospital Santa Bárbara, Complejo Hospitalario de Soria, Av. Santa Bárbara s/n 42002, Soria, Spain.
(Email: tnebreda@hsor.sacyl.es)

Since the identification of *B. burgdorferi* as the agent of Lyme disease in 1982, 11 tick-borne pathogenic bacteria have been described in Europe: *B. burgdorferi sensu lato* (*s.l.*), *Anaplasma* (*Ehrlichia*) *phagocytophilum* [5], *R. conorii*, *R. helvetica*, *Borrelia* spp. A14S, *R. mongolotimonae*, *R. slovaca*, *Borrelia* sp. nov., *B. hispanica*, *R. conorii* Israel and *R. conorii* Astrakhan [6]. All of them represent a threat to the health of human beings bitten by ticks.

Knowing which tick species parasitize humans, their life-cycles, and tick infection rates with human pathogens is essential for assessing the risks to public health and for adopting measures to prevent infection. Insight into the tick species that bite humans in a particular area can be obtained indirectly, either by the demonstration of anti-tick saliva antibodies in human serum [7–9] or by harvesting ticks from their resting places; vegetation, burrows, etc. [10–12] and animal hosts in that area [11–13]. However, it is more reliable to obtain this information directly by removing and identifying the ticks feeding on people [14–19]. In this way the ticks can also be screened for pathogens by a PCR-based method.

Here, we report the results of a 5-year study of tick-infested patients who attended health-care centres of the province of Soria, Spain. All their ticks were removed, identified and DNA extracts examined by PCR for the presence of pathogens.

MATERIALS AND METHODS

The province of Soria is located in the central–northern part of the Iberian Peninsula at a mean altitude of 1000 m. Its climate is continental, with cold dry winters and mild summers. It has extensive forested areas (*Pinus* spp., *Quercus* spp., *Juniperus* spp.) and a ground cover that serves as refuge for many species of wild (deer, foxes, hares, rats, mice, moles) and domestic (sheep, goats, dogs, cattle) animals. Human beings come into in contact with ticks through both professional (the population is predominantly rural) and recreational activities (hunting, fishing, picnicking, hiking).

From January 1997 to December 2001, ticks were collected from every patient who sought medical advice at the health-care centres of the province of Soria and at the Emergency Service of the Complejo Hospitalario de Soria after they had observed one or more ticks attached to some part of their bodies. A medical practitioner removed the ticks with tweezers, avoiding damaging the acarids. The tweezers were inserted between the skin of the patient and the mouthparts of the tick, exerting a gentle but continuous perpendicular traction away from the skin. The ticks were never handled manually nor impregnated with oil or any other similar substance.

The ticks were placed in a vial containing gauze moistened with physiological saline and were sent to the Territorial Social Welfare Service of Soria, and then to the Department of Parasitology of the School of Pharmacy of the University of Salamanca for identification and later detection of the bacterial species present in them.

The ticks collected were identified under a binocular lens following the keys for adult and immature forms referred to by Encinas-Grandes [20]. Once identified, the DNA from each tick was extracted and analysed by PCR for the following microorganisms: *B. burgdorferi s.l.*, *A. phagocytophilum* and *Rickettsia* spp. In the ticks collected from April 1998 onwards, the presence of *Francisella tularensis* was also explored.

For DNA extraction, ticks were first decontaminated by sequential washing in 45% alcohol, 30% alcohol and ultrapure water. After this, each tick was transferred to an individual test tube where it was bisected with a sterile blade and its DNA extracted in 500 μl of 5% Chelex-100 (Bio-Rad, Hercules, CA, USA), following the procedure described by Guttman et al. [21]. Each DNA sample was subsequently used as template in PCR analyses.

Amplification of the target sequences was carried out using primers and conditions previously described. For *B. burgdorferi s.l.*, the 5S–23S rRNA intergenic spacer was targeted using the method of Postic et al. [22]. The detection of *Rickettsia* spp. was by amplification of the citrate synthase gene (*gltA*) using the procedure of Regnery et al. [23] and, for rickettsia-positive samples, detection of spotted fever rickettsia was attempted by amplification of the *rOmpA* gene according to Roux et al. [24]. *A. phagocytophilum* was detected using the method of Goodman et al. [25], which targets a 151-bp fragment from the 16S rRNA gene. Finally, *F. tularensis* was detected using the method of Sjöstedt et al. [26], by amplification of the TUL4 lipoprotein gene.

Positive and negative controls were included in all PCR runs. The positive controls were respectively, DNA from *B. burgdorferi* strain Esp-1, *R. conorii*, *A. phagocytophilum* and *F. tularensis* subsp. *palaearctica*. As negative controls, both ultrapure water and DNA from ticks free of the above pathogens

(ticks from laboratory cultures) were incorporated in each run. PCR results were analysed in 1% or 2% agarose gels containing ethidium bromide, depending on the size of the expected band.

The PCR products were purified (QIAquick PCR purification kit, Qiagen, Hilden Germany) and sequenced in a fluorescence-based automated sequencing system (ABI 377 DNA sequencer; PerkinElmer Instruments, Norwalk, CT, USA) with the same primer set used in the amplification. The sequences generated were compared with those available in the databases (GenBank) using the BLASTn 2.0 search program in order to confirm the identity of the genospecies or species present.

Sample DNA extraction, amplification and, finally, analysis of the PCR product were performed in different rooms to prevent contamination.

RESULTS

In total, 185 ticks were collected from 179 individuals: 47 in 1997; 57 in 1998; 39 in 1999; 21 in 2000 and 21 in 2001.

The distribution of the ticks by species was: 103 (55·7%) *Dermacentor marginatus*; 23 (12·4%) *Ixodes ricinus*; 22 (11·9%) *Rhipicephalus bursa*; 16 (8·6%) *Haemaphysalis punctata*; 14 (7·6%) *Hyalomma marginatum*; 3 (1·6%) *Rhipicephalus sanguineus*; 2 (1·1%) *Argas reflexus*; 1 (0·5%) *Rhipicephalus turanicus* and 1 (0·5%) *Dermacentor reticulatus*. Of these, 97 ticks were female, 66 were male, 21 were nymphs, and one was a larva. Table 1 shows the ticks arranged by species, developmental stage, and the season they were collected.

No *F. tularensis* DNA was detected in any tick. Nor were there any cases of co-infection in ticks with more than one pathogen. Table 2 shows the pathogens detected within ticks. Sequencing and comparison of the *gltA* and *ompA* PCR products allowed *Rickettsia* spp. identification of 18 of the 26 positive tick samples. Eleven samples were *R. slovaca*: 10 of them (from 10 *D. marginatus*) shared 99·7–100% sequence similarity with the homologous fragment of the *gltA* gene of *R. slovaca* (GenBank, U59725), and the one detected in the *D. reticulatus* male tick shared 100% sequence identity with the homologous fragment of the *rOmpA* gene from *R. slovaca* (GenBank, U433808).

Six samples (from six *D. marginatus*) were identified as the *Rickettsia* spp. designated as RpA4 and DnS14. Since these two genotypes show similar sequences in the *gltA* gene fragment analysed, we were unable to determine which of them our six samples belonged to as they shared the same level of identity (between 97·1 and 100%) with the homologous fragment of both genotypes: RpA4 (GenBank, AF120029) and DnS14 (AF120028).

One sample (detected in a *R. sanguineus* tick) was identified as *R. massiliae* or *Rickettsia* spp. Bar29, as

Table 1. *Tick specimens removed from people living in the province of Soria (Spain) from 1997 to 2001: distribution by species, developmental stage and season in which they were collected*

	Winter	Spring	Summer	Autumn	Total
D. marginatus					
Nymph	—	—	3	—	3
Female	15	11	5	26	57
Male	7	5	10	21	43
Total	22	16	18	47	103
I. ricinus					
Larva	—	1	—	—	1
Nymph	—	7	3	5	15
Female	—	3	4	—	7
Total	0	11	7	5	23
R. bursa					
Female	—	7	6	—	13
Male	—	6	3	—	9
Total	0	13	9	0	22
H. punctata					
Female	2	5	—	4	11
Male	—	1	1	3	5
Total	2	6	1	7	16
Hyalomma marginatum					
Nymph	—	—	1	—	1
Female	—	3	2	—	5
Male	—	6	2	—	8
Total	0	9	5	0	14
R. sanguineus					
Nymph	1	—	—	—	1
Female	—	—	2	—	2
Total	1	0	2	0	3
Argas reflexus					
Nymph	1	—	—	—	1
Female	1	—	—	—	1
Total	2	0	0	0	2
D. reticulatus					
Male	—	1	—	—	1
Total	0	1	0	0	1
R. turanicus					
Female	—	1	—	—	1
Total	0	1	0	0	1
Total	27	57	42	59	185

Table 2. *Ticks* (*species and number of specimens*) *infected with the different agents* (*detected by PCR*)

	Rickettsia spp.	*A. phagocytophilum*	*B. burgdorferi s.l.*
D. marginatus	10 *R. slovaca* 6 RpA4/DnS14 7 unidentified	4	2
I. ricinus		1	
Hyalomma marginatum	1 *R. aeschlimannii*		
R. bursa		1	
R. sanguineus	1 Bar 29 or *R. massiliae*		
H. punctata		1	
D. reticulatus	1 *R. slovaca*		
Total	26	7	2

its *gltA* gene fragment shared 100% sequence identity with the homologous region of these two species (GenBank, U59720 and U59719). For the remaining eight samples positive for *Rickettsia* spp. by PCR (detected in 7 *D. marginatus* and in 1 *H. marginatum* ticks), the sequences of the PCR products could not be obtained and they, therefore, remained unidentified. The 26 ticks in which *Rickettsia* spp. were detected were all adults: 14 males and 12 females.

The two *D. marginatum* in which *B. burgdorferi s.l.* was detected were males. In both cases, sequencing of the PCR product failed and the genospecies present could not be identified.

The seven ticks infected with *A. phagocytophilum* included a nymph and six adults (2 males and 4 females). In all seven cases the 151-bp PCR product shared 100% identity with the homologous fragment of the 16S rRNA gene shared by *A. phagocytophilum* (GenBank M73220), *Ehrlichia equi* (M73223), and the HGE agent (U02521), now considered to be the same species, *A. phagocytophilum* [5].

DISCUSSION

Although our study was carried out over 5 years, the number of ticks removed from people was rather low (185). This is probably because Soria is the least populated province in Spain (nine residents per km^2) and, consequently, the population involved in the study was itself very small (92 396 residents). Even so, the mean annual rate of tick-bites recorded by us (40 ± 15 tick-bites per 100 000 residents) was notably lower than that obtained by Manfredi et al. [18] in a similar study (250 tick-bites per 100 000 residents) in Liguria, Italy.

The most frequent tick species identified in our study was *D. marginatus*, representing 55·7% of all the specimens collected, probably because Soria provides an excellent habitat for *D. marginatus* (steppe area, temperate forest, meadows and grazing land). It was also the species infected with the greatest variety of pathogens. Among these, the most frequent was *R. slovaca*, the TIBOLA (Tick-borne lymphadenopathy)-producing agent, for which *D. marginatus* and *D. reticulatus* are the main vectors [27, 28]. *R. slovaca* was detected for the first time in Spain by Oteo et al. [29, 30] in the province of La Rioja, which is adjacent to Soria. Since then, more than 20 cases of TIBOLA have been diagnosed in that area of Spain [31]. This and the high *R. slovaca* infection rate detected by us in *D. marginatus* (9·7%) suggests that in this area (Soria and La Rioja) there may be an active focus of TIBOLA transmission. The other two rickettsias identified in *D. marginatus* were the genotypes RpA4 and DnS14, which are almost identical to each other and, in turn, are very close to the group *R. massiliae*/Bar29 [32]. The genotypes RpA4 and DnS14 seem to be quite abundant in the *D. marginatus* populations of Soria, but their pathogenicity for humans is not yet clear.

The finding of *A. phagocytophilum* in *D. marginatus* was noteworthy as this bacterium is usually transmitted by ticks from the *I. ricinus* complex [33], and it has not previously been reported in *D. marginatus*. However, *A. phagocytophilum* has been previously detected not only in *I. ricinus* but in *H. punctata*, *H. inermis*, *R. bursa* and *D. reticulatus* in Spain (although not in other countries) [34], and we also found *A. phagocytophilum* in *I. ricinus*, *R. bursa* and *H. punctata*. It is not known whether or not all these

tick species are true vectors of *A. phagocytophilum*, but all the patients in this study were free of infection and so could not have been the source of the agent. This suggests that the ticks became infected with *A. phagocytophilum* transtadially [35], in which case they could well be true vectors for this pathogen.

D. marginatus was also the only tick species in which we detected DNA from *B. burgdorferi s.l.* As *D. marginatus* is an anthropophilic tick and can become infected by *B. burgdorferi*, as supported by Angelov et al. [36], it may be a vector for Lyme disease.

Ticks of the *I. ricinus* complex are the main vectors of Lyme disease throughout the world [1, 37], and in many areas of Europe it is almost the only tick that bites humans [3, 4, 18, 38–41]. Surprisingly, in our study it only represented 12·4% of all the ticks found, being the second most frequent after *D. marginatus*. Contrary to the observations of other authors [41, 42], who have reported high rates of *B. burgdorferi*-infected *I. ricinus*, no Lyme borrelia was detected in this study in any of the *I. ricinus* specimens. The 23 *I. ricinus* ticks examined in this study is a very low number to make any generalizations about the importance of this species as a vector of *B. burgdorferi s.l.* in this area. However, the small number of *I. ricinus* found on humans, plus the absence of *B. burgdorferi s.l.* in them, is consistent with only one case of Lyme disease being diagnosed in Soria during the 5-year study.

R. sanguineus represented only 1·6% of all the ticks collected in the present work, and in most geographical areas of its range, humans are only occasional hosts of this tick species [14–16, 19, 43–45]. *R. sanguineus* is a relatively host-specific tick, preferring dogs to other mammals [11, 12, 18, 46], and consequently tends only to feed on humans when no dogs are available [1, 12, 46–48]. We did not find *R. conorii* in any of the three *R. sanguineus* specimens collected, but we found *Rickettsia* spp. Bar29 – or the very similar *R. massiliae* – in one. The patient bitten by that tick did not develop spotted fever. Like Genchi [49] and Kelly et al. [50], we believe that owing to its specificity for dogs *R. sanguineus* is not a very efficient vector of zoonoses.

As expected [14, 16, 17], most of the ticks found in the present study were adults (88·1%). This trend was observed in all species except *I. ricinus*, whose nymphs were more anthropophilic than were adults. Such a difference may be due to nymphs and larvae often feeding on smaller animals, such as rodents, whereas the adult ticks prefer larger hosts, such as dogs, cats, goats and sheep [1, 13]. Furthermore, the adult ticks are large enough to be readily visible and thus removed, while nymphs and larvae may pass unnoticed and, additionally, their bite is not usually very painful [1]. Like Slaff & Newton [16] and Felz et al. [17], we also found a greater number of female than male ticks.

In Soria, ticks were found to feed on people throughout the year, although the seasons with the highest tick-bite risk were spring (when most tick species are maximally active) and autumn (due to the high activity of *D. marginatus*, which is the most anthropophilic species in this area). The high number of species found feeding on humans is remarkable: eight ixodids and one argasid. In Liguria (Italy) only three tick species were found feeding on humans [18], and in central Europe humans are bitten almost exclusively by *I. ricinus* [4, 39]. Thus, in the province of Soria, people are bitten by a higher number of species than in most other European countries, and this may expose them to a greater variety of pathogens [1].

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. **Camicas JL, Hervy JP, Adam F, Morel PC.** The ticks of the world (Acarida, Ixodida). Paris: Orstom ed., 1998.
3. **Doan-Wiggins L.** Tick-borne diseases. Emerg Med Clin North Am 1991; **9**: 303–325.
4. **Estrada Peña A, Jongejan F.** Ticks feeding on humans: a review of records on human-biting *Ixodoidea* with special reference to pathogen transmission. Exp Appl Acarol 1999; **23**: 685–715.
5. **Dumler JS, Barbet AF, Bekker CP, et al.** Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol 2001; **51**: 2145–2165.
6. **Parola P, Raoult D.** Tick-borne bacterial diseases emerging in Europe. Clin Microbiol Infect 2001; **7**: 80–83.
7. **Schwartz BS, Goldstein MD, Childs JE.** Antibodies to *Borrelia burgdorferi* and tick salivary gland proteins in New Jersey outdoor workers. Am J Public Health 1993; **83**: 1746–1748.
8. **Pérez-Sánchez R, Oleaga-Pérez A, Encinas-Grandes A.** Analysis of the specificity of the salivary antigens of *Ornithodoros erraticus* for the purpose of serological detection of swine farms harbouring the parasite. Parasite Immunol 1992; **14**: 201–216.

9. **Sanders ML, Scott AL, Glass GE, Schwartz BS.** Salivary gland changes and host antibody responses associated with feeding of male lone star ticks (Acari: Ixodidae). J Med Entomol 1996; **33**: 628–634.
10. **Lane RS.** Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. Am J Trop Med Hyg 1996; **55**: 165–173.
11. **Lavender DR, Oliver JH.** Ticks (Acari: Ixodidae) in Bulloch County, Georgia. J Med Entomol 1996; **33**: 224–231.
12. **Magnarelli LA, Andreadis TG, Stafford III KC, Holland CJ.** *Rickettsiae* and *Borrelia burgdorferi* in Ixodid ticks. J Clin Microbiol 1991; **29**: 2798–2804.
13. **Mumcuoglu KY, Frish K, Sarov B, et al.** Ecological studies on the brown dog tick *Rhipicephalus sanguineus* (Acari: Ixodidae) in southern Israel and its relationship to spotted fever group *Rickettsiae*. J. Med Entomol 1993; **30**: 114–121.
14. **Smith RP, Lacombe EH, Rand PW, Dearborn R.** Diversity of ticks species biting humans in an emerging area for Lyme disease. Am J Public Health 1992; **82**: 66–69.
15. **Guglielmone AA, Mangold AJ, Viñabal AE.** Ticks (Ixodidae) parasitizing humans in four provinces of north-western Argentina. Ann Trop Med Parasitol 1991; **85**: 539–542.
16. **Slaff M, Newton NH.** Location of tick (Acari: Ixodidae) attachment sites on humans in North Carolina. J Med Entomol 1993; **30**: 485–488.
17. **Felz MW, Durden LA, Oliver JH.** Ticks parasitizing humans in Georgia and South Carolina. J Parasitol 1996; **82**: 505–508.
18. **Manfredi MT, Dini V, Piacenza S, Genchi C.** Tick species parasitizing people in an area endemic for tick-borne diseases in North-Western Italy. Parassitologia 1999; **41**: 555–560.
19. **Felz MW, Durden LA.** Attachment sites of four tick species (Acari: Ixodidae) parasitizing humans in Georgia and South Carolina. J Med Entomol 1999; **36**: 361–364.
20. **Encinas-Grandes A.** Ticks of the province of Salamanca (central/NW Spain). Prevalence and parasitization intensity in dogs and domestic Ungulates. Ann Parasitol Hum Comp 1986; **61**: 95–107.
21. **Guttman DS, Wang PW, Wang IN, Bosler EM, Luft BJ, Dykhuizen DE.** Multiple infections of *Ixodes scapularis* ticks by *Borrelia burgdorferi* as revealed by single-strand conformation polymorphism analysis. J Clin Microbiol 1996; **34**: 652–656.
22. **Postic D, Asous MV, Grimont PAD, Baranton G.** Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf(5S)-rrl(23S) intergenic spacer amplicons. Int J Syst Barcteriol 1994; **44**: 743–752.
23. **Regnery RL, Spruill CL, Plykaitis BD.** Genotypic identification of rickettsiae and estimation of intra-species sequence divergence for portions of two rickettsial genes. J Bacteriol 1991; **173**: 1576–1589.
24. **Roux V, Fournier PE, Raoult D.** Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J Clin Microbiol 1996; **34**: 2058–2065.
25. **Goodman JL, Nelson C, Vitale B, et al.** Direct cultivation of the causative agent of human granulocytic ehrlichiosis. N Engl J Med 1996; **4**: 209–215.
26. **Sjöstedt A, Erikson U, Bernglud L, Tarnvik A.** Detection of *Francisella tularensis* in ulcers of patients with tularemia by PCR. J Clin Microbiol 1997; **35**: 1045–1048.
27. **Lakos A.** Tick-borne lymphadenopathy – a new rickettsial disease? Lancet 1997; **350**: 1006–1007.
28. **Raoult D, Berbis P, Roux V, Xu W, Maurin M.** A new tick-transmitted disease due to *Rickettsia slovaca*. Lancet 1997; **350**: 112–113.
29. **Oteo JA, Blanco JR, Martínez de Artola V, Anda P.** Erythema *migrans*-like lesions after *Dermacentor* sp. Tick-bite without evidence of *Borrelia burgdorferi* infection. In: International Conference on Rickettsia and Rickettsial Diseases and American Society for Rickettsiology, 14th Sesquiannual Joint Meeting. Marsella, France, 13–16 June 1999.
30. **Oteo JA, Blanco JR, Ibarra V, Martínez de Artola V.** Eschar, erythema and lymphadenopathy for *Dermacentor marginatus*. A new tick-borne disease. Enferm Infecc Microbiol Clin 2000; **18** (Suppl 1): 133.
31. **Oteo JA, Ibarra V.** DEBONEL (Dermacentor-borne-necrosis-erythema-lymphadenopathy). A new tick-borne disease? Enferm Infecc Microbiol Clin 2002; **20**: 51–52.
32. **Rydkina E, Roux V, Rudakov N, et al.** New Rickettsiae in ticks collected in territories of the former Soviet Union. Emerg Infect Dis 1999; **5**: 811–814.
33. **Blanco JR, Oteo JA.** Human granulocytic ehrlichiosis in Europe. Clin Microbiol Infect 2002; **8**: 763–772.
34. **Barral-Lahidalga M.** Estudio de la infección por *Borrelia burgdorferi*, grupo *Ehrlichia phagocytophila* y virus de la encefalitis ovina en las poblaciones de ixódidos de la comunidad autónoma vasca. Doctoral Thesis. University of Zaragoza. Spain, 1998.
35. **Parker RJ.** The Australian brown dog tick *Rhipicephalus sanguineus* as an experimental parasite of cattle and vector of *Anaplasma marginale*. Aust Vet J 1982; **58**: 47–50.
36. **Angelov L, Dimova P, Berbencova W.** Clinical and laboratory evidence of the importance of the tick *D. marginatus* as a vector of *B. burgdorferi* in some areas of sporadic Lyme disease in Bulgaria. Eur J Epidemiol 1996; **12**: 499–502.
37. **Wang G, Van Dam AP, Schwartz I, Dankert J.** Molecular typing of Borrelia burgdorferi sensu lato: taxonomic, epidemiological and clinical implications. Clin Microbiol Rev 1999; **12**: 633–653.
38. **Paul H, Gerth H, Ackermann R.** Infectiousness for humans of *Ixodes ricinus* containing *Borrelia burgdorferi*. Zbl Bakt Hyg 1986; **263**: 473–476.

39. **Maiwald M, Oehme R, March O, et al.** Transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in southwest Germany. Epidemiol Infect 1998; **121**: 103–108.
40. **Junttila J, Peltomaa M, Soini H, Marjamaki M, Viljanen MK.** Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks in urban recreational areas of Helsinki. J Clin Microbiol 1999; **37**: 1361–1365.
41. **Robertson JN, Gray JS, Stewart P.** Tick bite and Lyme borreliosis risk at a recreational site in England Eur J Epidemiol 2000; **16**: 647–652.
42. **Mejlon HA, Jaenson TGT.** Seasonal prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* in different vegetation types in Sweden. Scand J Infect Dis 1993; **25**: 449–456.
43. **Mejlon HA, Jaenson G.** Aggiornamenti sulle zecche d'Italia. Loro distribuzione e sistema I. G. M. Bonifica 1988; **4** (Suppl 1): 6–11.
44. **Kardtzke JT, Neidhardt K, Dzuban DP, Sanchez JL, Azad AF.** Cluster of tick-borne infections at Fort Chaffee, Arkansas: *Rickettsiae* and *Borrelia burgdorferi* in *Ixodid* ticks. J Med Entomol 1992; **29**: 669–672.
45. **Smith-Fiola DC, Hallman WK.** Tick bite victims and their environment: the risk of Lyme disease. NJ Med 1995; **92**: 601–603.
46. **Bernasconi MV, Valsangiacomo C, Balmelli T, Peter O, Piffaretti JC.** Tick zoonosis in the southern part of Switzerland (Canton Ticino): occurrence of *Borrelia burgdorferi* sensu lato and *Rickettsia* sp. Eur J Epidemiol 1997; **13**: 209–215.
47. **Goddard J.** Focus of human parasitism by the brown dog tick, *Rhipicephalus sanguineus* (Acari: Ixodidae). J Med Entomol 1989; **26**: 628–629.
48. **Carpenter TL, McMeans MC, McHugh CP.** Additional instances of human parasitism by the brown dog tick (Acari: Ixodidae). J Med Entomol 1990; **27**: 1065–1066.
49. **Genchi C.** Arthopoda as zoonoses and their implications. Vet Parasitol 1992; **44**: 21–33.
50. **Kelly PJ, Mason PR.** Tick-bite fever in Zimbabwe. S Afr Med J 1991; **80**: 233–236.