Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Detection of Tick Borne Zoonotic Bacteria by PCR in Dogs

Şükrü KIRKAN[1*] , Göksel ERBAŞ[1] , Hafize Tuğba YÜKSEL DOLGUN[1] ,
Yiğit SEFEROĞLU[1] , Uğur PARIN[1] , Serap SAVAŞAN[1]

[1]Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Aydın, TÜRKİYE

A B S T R A C T

The prevalence of some tick-borne zoonotic bacteria (*Borrelia* sp., *Coxiella burnetii*, and *Rickettsia* sp.) in dog blood samples were examined using by PCR. A total of 200 dog blood samples were collected from dogs. Three pathogens for dogs, *Borrelia burgdorferi*, *C. burnetii* and *Rickettsia* sp. of were detected in dog blood samples. A single step PCR was performed for the *C. burnetii* and multiplex PCR was applied for the *Rickettsiae* spp. and *Borrelia burgdorferi*. At the end of PCR study, *Borrelia burgdorferi*, *C. burnetii* and *Rickettsiae* sp. target pathogens were identified at the rate of 55.0% from all blood samples. The percentage distributions according to identified species were at the rate of *C. burnetii* 1.5%, *Borrelia burgdorferi* sensu lato 2.0% and *Rickettsiae* spp. 51.5%. The results indicated that dogs may play a role in disseminating tick-borne zoonotic bacteria, thereby posing a potential health hazard to humans.
Keywords: Tick-borne zoonotic bacteria, identification, multiplex PCR.

Köpeklerde Kene Kaynaklı Zoonotik Bakterilerin PCR Yöntemiyle Belirlenmesi

Ö Z E T

Araştırmamızda, köpek kan örneklerinde bazı kene kökenli zoonotik bakterilerin prevalansı PCR metodu kullanılarak araştırıldı. Araştırmamızda 200 adet köpek kan örneği kullanıldı. Köpekler için patojen oldukları bilinen *Borrelia burgdorferi*, *C. burnetii* ve *Rickettsiae* sp. varlığı köpek kan örneklerinde araştırıldı. *C. burnetii için tek adımlı PCR ve Rickettsiae* sp. ile *Borrelia burgdorferi* için multipleks PCR uygulandı· PCR işlemi sonucunda, tüm kan örneklerinin %55·0'inden *Borrelia burgdorferi*, *C. burnetii* and *Rickettsiae* spp. identifiye edildi. İdentifiye edilen türlerin %1,5'i *C. burnetii*, %2'si *Borrelia burgdorferi* sensu lato ve %51,5'i *Rickettsiae* spp. olarak dağılım gösterdiği saptandı. Araştırmanın sonuçları, köpeklerin kene kökenli zoonotik bakterilerin yayılımında bir risk faktörü olabileceğini göstermektedir. Kene kökenli zoonotik bakteriler insan sağlığı açısından bir tehlike oluşturabilmektedir.
Anahtar Kelimeler: Kene kaynaklı zoonotik bakteriler, identifikasyon, multipleks PCR.

Kırkan et al.   Tick Borne Zoonotic Bacteria in Dogs

41

Introduction

Ticks are the most of great importance vectors of pathogenic bacteria, viruses, and protozoa and causing diseases in animals and humans worldwide. Most of those agents are accepted as both bioterrorism (such as *C. burnetii*) and vector-borne pathogens (Azad and Radulovic, 2003). In addition, *Rickettsia* and some *Borrelia* species also cause zoonotic diseases reported in both animals and humans (Piesman and Gern, 2004; Parola et al., 2005).

Ticks are the most important vectors of microorganisms, and can transmit a much larger number of pathogenic microorganisms to both animals and humans than any other arthropod group. Ticks can adapt to climate changes caused by global warming and related regions. Ticks are epidemiological markers for the infectious agent (Psaroulaki et al., 2006).

By sticking to their hosts, ticks ensure that infectious agents are effectively transmitted easily and that microorganisms spread to different geographical habitats through the pets they travel on. Pathogens that are ingested by ticks can spread interstellar or interovary. Because female ticks are highly fertile and this accelerates the spread of infectious agents in pet dogs and their owners. However, a small number of tick species can transmit infectious agents to pet dogs and their owners (Hillyard, 1996).

Tick borne infectious diseases in dogs are babesiosis and ehrlichiosis. However, *Borrelia burgdorferi* and *Rickettsia conorii* generally cause subclinically infections, and in this case, it is quite difficult to assess their relationship with clinical disease in dogs (Levy and Magnarelli, 1992). Dogs are responsive to infection by *Coxiella burnetii* (Weissenbock and Holtzmann, 1996). The natural cycle of the transmission of this agent the dog is play a role in infected dogs (Senneville et al., 1991).

Ticks can a reservoirs of spotted fever group (SFG) rickettsiae. Pathogens of the SFG rickettsiae are usually associated with ixodid ticks. The ixodid ticks are transfer SFG ricketsiae to vertebrates via salivary secretions both transtadially and transovarially (Raoult and Roux, 1997)

Coxiella burnetii is obligate intracellular bacteria and they live in the phagolysosomes of the host cell. The ticks carry *C. burnetii* both transovarially and transtadially and are a reservoir for this pathogen. It has been reported that the role of ticks in the transmission of *C. burnetii*, which is found in several tick species, to humans is minimal. However, it is stated that the excrement of ticks carrying the *C. burnetii* agent is much more important in transmitting the agent to humans (Parola and Raoult, 2001).

Borrelia burgdorferi sensu lato is caused canine borreliosis and it's transmitted by ticks of the genus Ixodes. (Filipuzzi-Jenny et al., 1993). The distribution of borreliosis is increasing rapidly in Northern Europe and it is reported that *I. ricinus* ticks transmit the agent to humans in urban areas (Talleklint and Jaenson, 1998; Junttila et al., 1999). There is significant genetic heterogeneity between European and North American isolates of *B. burgdorferi* (Lovrich et al., 1994). *B. burgdorferi* sensu lato, *B. japonica*, *B. afzelii*, *B. garinii* genotypes cause diseases in humans in the northern hemisphere (Filipuzzi-Jenny et al., 1993). The extent to which *B. japonica*, *B. afzelii*, *B. garinii* contribute to dogs' infection is unclear (Azuma et al., 1994).

PCR methods are widely used as rapid and effective tools for the identification of tick-borne pathogens. Sensitivity-specificity is significant in identifying tick-borne infections with PCR-based methods (Rijpkema et al., 1995; Schouls et al., 1999).

The aim of this study was to evaluate the prevalence of zoonotic tick-borne pathogens in dogs Western Türkiye.

Materials and Methods

Sampling

Dog blood samples (n=200) were collected from dogs

Table 1. The sampling provinces and distribution of dog breed.

Dog Breeds	Sampling Provinces		
	Aydın	İzmir	Muğla
Mongrel (n=73)	42	24	7
Kangal (n=35)	14	8	13
Setter (n=22)	6	7	9
Terrier (n=20)	5	6	9
Pointer (n=20)	5	7	8
German Shepherd (n=18)	6	4	8
Doberman (n=12)	2	4	6
Total	**80**	**60**	**60**

DNA Extraction from dog blood samples

Kırkan et al.   Tick Borne Zoonotic Bacteria in Dogs

42

located in western Türkiye. The numerical distribution of the dog blood samples is shown in Table 1. For the dog blood samples, the researchers were taken permission from Adnan Menderes University Local Ethical Committee of Animal Experiments (Document No: 2009/54).

DNA isolation from dog blood samples was done with a DNA extraction kit. (Fermentas®) as recommended by the manufacturer. DNA's were stored at -20°C until PCR studies.

Primers and PCR

Coxiella burnetii (Trans 1-Trans 2), SFG Rickettsiae (Rr190.70p-Rr190.602n), *Borrelia* sp. (BORF-16S) and *B. burgdorferi* sensu lato (23SN2-5SCB) primers were designed which informed as Barandika et al. (2007).

PCR conditions of *C. burnetii*, SFG Rickettsiae and *Borrelia* sp. were given by Barandika et al. (2007).

For the *C. burnetii* the PCR was performed according to Barandika et al. (2007). The reference strain of *C. burnetii* Nine Mile Strain Phase I was used as positive control and *Escherichia coli* was also used to negative control for the PCR.

For the detection of SFG Rickettsiae and *Borrelia* spp., the multiplex PCR was examined according to Barandika et al. (2007).

Detection of the Amplified Products

PCR amplicon were detected by agarose gel electrophoresis and were visualised using the Vilber Lourmat Gel Documentation System (Vilber Lourmat, Germany).

Results

In this study, a total of 200 dogs blood samples collected from Aydın, İzmir and Muğla provinces were examined by using PCR for the diagnosis of Rickettsiosis, Borreliosis and Q Fever infections from tick-borne diseases in dogs. DNA samples were extracted from the blood samples and then PCR was performed on all DNA samples. Because of the differences between the binding scores in the PCR process, the studies are step-by-step.

In the single step PCR for the *C. burnetii* were found to be positive 3 (1.5%) samples obtained from 200 different dog breeds blood samples. It was determined that *C. burnetii* was positive in 1 Kangal dog in Izmir province and 2 Pointer dogs in Muğla province. *C. burnetii* positivity was not found in 80 dog blood samples taken from Aydın province (Figure 1).

As a result of the multiplex PCR for *Rickettsiae* sp. and *Borrelia* sp., a total of 4 (2.0%) samples were found to be positive for the *Borrelia* sp. and 103 (51.5%) samples were found to be positive for the *Rickettsiae* sp. The distribution of *Rickettsiae* sp. and *Borrelia* sp.
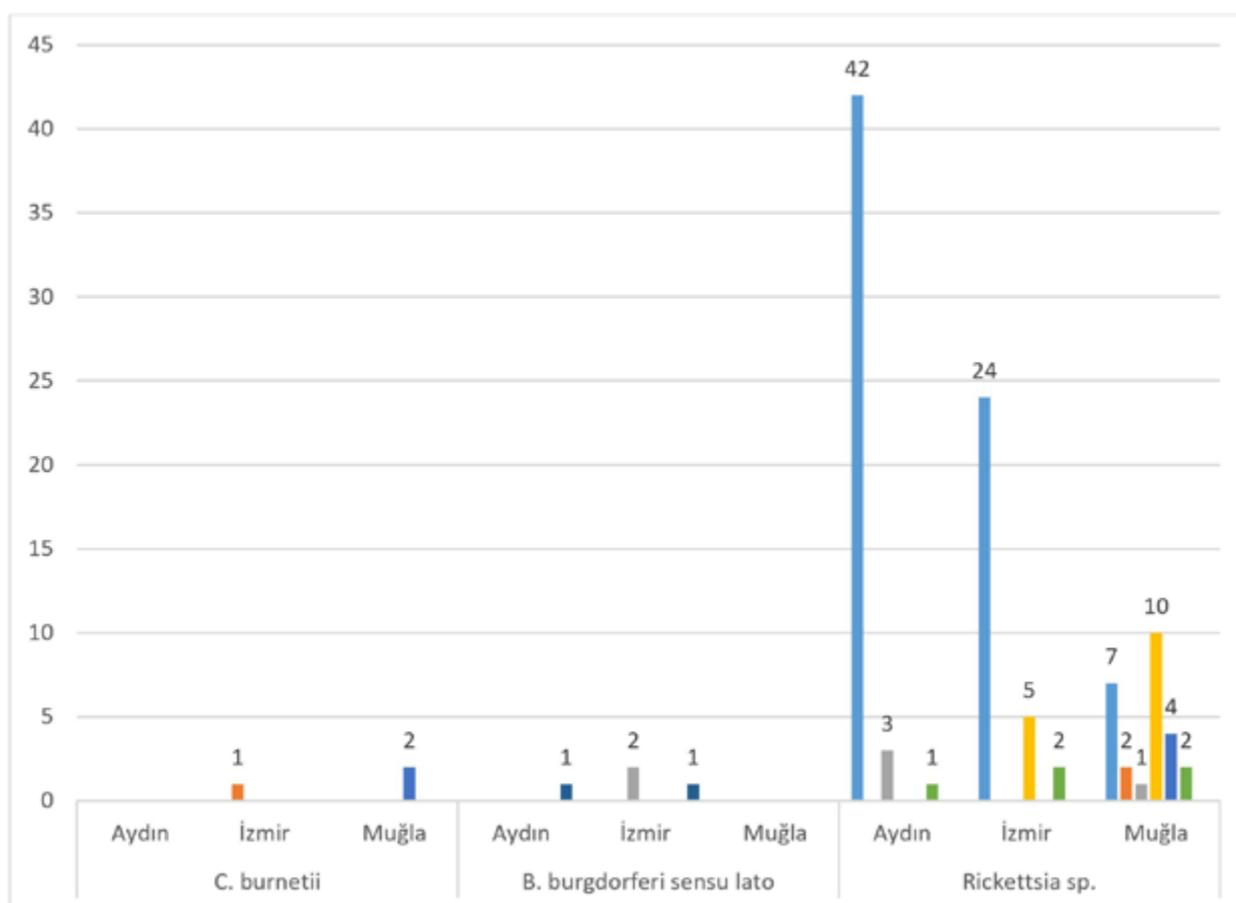

Figure 1. Distribution of PCR positive tick-borne zoonotic bacteria by dog breeds and regions

positive samples by dog breeds is shown in Figure 1. It is noteworthy that all blood samples of 73 Mongrel dogs collected in our study were *Rickettsia* sp. positive.

In this study, PCR tests with DNA from 200 blood samples taken from dogs resulted in 1.5% *C. burnetii*, 2.0% *B. burgdorferi* sensu lato and 51.5% *Rickettsiae* sp. positivity were determined (Figure 1).

In this study, *Rickettsia* sp. was found to be PCR positive at different rates in all regions, while *C. burnetii* in Aydın and *Borrelia burgdorferi* sensu lato in Muğla were not found to be PCR positive.

Discussion

Investigations in different parts of the world have found a close relationship between ticks and tick-borne pathogens (Magnarelli et al., 1995; Lane, 1996; Kramer et al., 1999; Kosik-Bogacka et al., 2004; Wielinga et al., 2006). To take into consideration the geographical and climatic conditions of Türkiye, the various types of ticks within our borders continue their existence. The ticks have completed a part of their life cycle in dogs. During this process, it is thought that dogs carry tick-borne bacterial zoonotic infections and infect people in various ways. Diseases such as Borreliosis and Ricketsiosis that were found to be among tick-borne infections have been reported to be subclinical form in dogs and the dogs are susceptible to *Coxiella burnetii* infections. Especially, it is argued that dogs play a role in the transmission of these infections to humans (Levy and Magnerelly, 1992; Mumcuoglu et al., 1993).

In our study, it was aimed to investigate the presence of *C. burnetii*, *B. burgdorferi* sensu lato and *Rickettsiae* sp. zoonotic bacteria in dogs, which are particularly threatening to human health. Our research materials consist of 200 blood samples taken from dogs.

Lyme Borreliosis is very common in the northern hemisphere. There are 60,000 cases reported in Europe and 15,000 cases reported in the United States each year (Steere, 2000; Hayes and Piesman, 2003). In some countries in Europe, the incidence of the disease is calculated to be 155 out of every 100,000 cases due to regional variability and diversity (Stanek et al., 2012). In some studies (Barral et al., 2002; Gil et al., 2005; Barandika et al., 2008), the presence of *B. burgdorferi* was also detected at low rates. In our study, 2% *B. burgdorferi* sensu lato was isolated in dogs.

Despite the fact that there are many species of tick in the world, it is seen that the rate of transmission of natural *C. burnetti* infection and humans is low (Maurin and Raoult, 1999). *Coxiella burnetii* is also isolated from several species of ticks in Europe (Rehacek et al., 1994; Psaroulaki et al., 2006; Smetanova et al., 2006). In our study, dog blood samples collected from western regions of our country were used and 1.5% of *C. burnetii* were found in dogs by PCR.

Rickettsia sp. was isolated from tick-borne infections frequently. In a survey of prevalence in South Brazil, *Rickettsia* sp. reported a positive rate of 33.7% in dogs (Saito et al., 2008) and reported a positive rate of 81.3% in Brazil's Minas Gerais area (Vianna et al., 2008). In a research conducted in northern Greece, the presence of *Rickettsia* sp. was demonstrated locally (Psaroulaki et al., 2006). In our study, *Rickettsia* sp. positivity was detected in 103 (51.5%) of 200 blood samples taken from dogs.

Conclusion

In conclusion, considering the density of tick populations in Türkiye, the importance of tick-borne bacterial zoonoses and their adaptation to the field conditions is becoming important. In addition to conventional methods, the development of fast and safe methods will ensure the detection of diseases in a short time and contribute to the country's economy with treatment and prophylactic measures to be made as an early diagnosis. In addition, infection of tick-borne bacterial zoonoses will be prevented and community health will be preserved.

Acknowledgements

Conflict of interest

The authors declare that they have no conflict of interest.

References

Azad, A. F., & Radulovic, S. (2003). Pathogenic rickettsiae as bioterrorism agents. *Annals of the New York Academy of Sciences*, *990*(1), 734-738. https://doi.org/10.1111/j.1749-6632.2003.tb07452.x

Azuma, Y., Isogai, E., Isogai, H., & Kawamura, K. (1994). Canine Lyme disease: clinical and serological evaluations in 21 dogs in Japan. *The Veterinary Record*, 134(15), 369-372. https://doi.org/10.1136/vr.134.15.369

Barandika, J. F., Hurtado, A., García-Esteban, C., Gil, H., Escudero, R., Barral, M., Jado I, Juste RA, Anda P., & García-Pérez, A. L. (2007). Tick-borne zoonotic bacteria in wild and domestic small mammals in northern Spain. *Applied and Environmental Microbiology*, 73(19), 6166-6171. https://doi.org/10.1128/AEM.00590-07

Barandika, J. F., Hurtado, A., García-Sanmartín, J., Juste, R. A., Anda, P., & García-Pérez, A. L. (2008). Prevalence of tick-borne zoonotic bacteria in questing adult ticks from northern Spain. *Vector-borne and Zoonotic Diseases*, 8(6), 829-836. https://doi.org/10.1089/vbz.2008.0023

Barral, M., Garcia-Perez, A. L., Juste, R. A., Hurtado, A., Escudero, R., Sellek, R. E., & Anda, P. (2002). Distribution of Borrelia burgdorferi sensu lato in Ixodes ricinus (Acari: Ixodidae) ticks from the Basque Country, Spain. *Journal of Medical Entomology*, 39(1), 177-184. https://doi.org/10.1603/0022-2585-39.1.177

Filipuzzi-Jenny, E., Blot, M., Schmid-Berger, N., Meister-Turner, J., & Meyer, J. (1993). Genetic diversity among Borrelia burgdorferi isolates: more than three genospecies?. *Research in Microbiology*, 144(4), 295-304. https://doi.org/10.1016/0923-2508(93)90014-S

Gil, H., Barral, M., Escudero, R., García-Pérez, A. L., & Anda, P. (2005). Identification of a new Borrelia species among small mammals in areas of northern Spain where Lyme disease is endemic. *Applied and Environmental Microbiology*, 71(3), 1336-1345. https://doi.org/10.1128/AEM.71.3.1336-1345.2005

Hayes, E. B., & Piesman, J. (2003). How can we prevent Lyme disease?. *New England Journal of Medicine*, 348(24), 2424-2430. https://doi.org/10.1056/NEJMra021397

Kırkan et al.   Tick Borne Zoonotic Bacteria in Dogs

44

Hillyard, P. D. (1996). Diseases carried by ticks in NW Europe: their medical and veterinary importance. *Ticks of North-West Europe. Synopses of the British Fauna (New Series). FSC Publications*, 22-23.

Junttila, J., Peltomaa, M., Soini, H., Marjamäki, M., & Viljanen, M. K. (1999). Prevalence of Borrelia burgdorferi in Ixodes ricinus ticks in urban recreational areas of Helsinki. *Journal of Clinical Microbiology*, 37(5), 1361-1365. https://doi.org/10.1128/JCM.37.5.1361-1365.1999

Kosik-Bogacka, D., Kuzna-Grygiel, W., & Bukowska, K. (2004). The prevalence of spirochete Borrelia burgdorferi sensu lato in ticks Ixodes ricinus and mosquitoes Aedes spp. within a selected recreational area in the city of Szczecin. *Annals of Agricultural and Environmental Medicine*, 11(1), 105-108

Kramer, V. L., Randolph, M. P., Hui, L. T., Irwin, W. E., Gutierrez, A. G., & Vugia, D. J. (1999). Detection of the agents of human ehrlichioses in ixodid ticks from California. *The American Journal of Tropical Medicine and Hygiene*, 60(1), 62-65. https://doi.org/10.4269/ajtmh.1999.60.62

Lane, R. S. (1996). Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. *The American Journal of Tropical Medicine and Hygiene*, 55(2), 165-173. https://doi.org/10.4269/ajtmh.1996.55.2.TM0550020165

Levy, S. A., & Magnarelli, L. A. (1992). Relationship between development of antibodies to Borrelia burgdorferi in dogs and the subsequent development of limb/joint borreliosis. *Journal of the American Veterinary Medical Association*, 200(3), 344-347.

Lovrich, S. D., Callister, S. M., Lim, L. C. L., DuChateau, B. K., & Schell, R. F. (1994). Seroprotective groups of Lyme borreliosis spirochetes from North America and Europe. *Journal of Infectious Diseases*, 170(1), 115-121. https://doi.org/10.1093/infdis/170.1.115

Magnarelli, L. A., Denicola, A., Stafford 3rd, K. C., & Anderson, J. F. (1995). Borrelia burgdorferi in an urban environment: white-tailed deer with infected ticks and antibodies. *Journal of Clinical Microbiology*, 33(3), 541-544. https://doi.org/10.1128/jcm.33.3.541-544.1995

Maurin, M.D.F., & Raoult D. (1999). *Q fever. Clinical Microbiology Reviews*, 12, 518-553. https://doi.org/10.1128/CMR.12.4.518

Mumcuoglu, K. Y., Frish, K., Sarov, B., Manors, E., Gross, E., Gat, Z., & Galun, R. (1993). Ecological studies on the brown dog tick Rhipicephalus sanguineus (Acari: Ixodidae) in southern Israel and its relationship to spotted fever group rickettsiae. *Journal of Medical Entomology*, 30(1), 114-121. https://doi.org/10.1093/jmedent/30.1.114

Parola, P., & Raoult, D. (2001). Tick-borne bacterial diseases emerging in Europe. *Clinical Microbiology and Infection*, 7(2), 80-83. https://doi.org/10.1046/j.1469-0691.2001.00200.x

Parola, P., Davoust, B., & Raoult, D. (2005). Tick-and flea-borne rickettsial emerging zoonoses. *Veterinary Research*, 36(3), 469-492. https://doi.org/10.1051/vetres:2005004

Piesman, J., & Gern, L. (2004). Lyme borreliosis in europe and North america. *Parasitology*, 129(1), 191-220. https://doi.org/10.1017/S0031182003004694

Psaroulaki, A., Ragiadakou, D., Kouris, G., Papadopoulos, B., Chaniotis, B., & Tselentis, Y. (2006). Ticks, tick-borne Rickettsiae, and Coxiella burnetii in the Greek island of Cephalonia. *Annals of the New York Academy of Sciences*, 1078(1), 389-399. http://dx.doi.org/10.1196/annals.1374.077

Raoult, D., & Roux, V. (1997). Rickettsioses as paradigms of new or emerging infectious diseases. *Clinical Microbiology Reviews*, 10(4), 694-719. https://doi.org/10.1128/CMR.10.4.694

Rehacek, J., Kaaserer, B., Urvölgyi, J., Lukáčová, M., Kováčová, E., & Kocianová, E. (1994). Isolation of Coxiella burnetii and of an unknown rickettsial organism from Ixodes ricinus ticks collected in Austria. *European Journal of Epidemiology*, 719-723. https://doi.org/10.1007/BF01719288

Rijpkema, S. G., Molkenboer, M. J., Schouls, L. M., Jongejan, F., & Schellekens, J. F. (1995). Simultaneous detection and genotyping of three genomic groups of Borrelia burgdorferi sensu lato in Dutch Ixodes ricinus ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. *Journal of Clinical Microbiology*, 33(12), 3091-3095. https://doi.org/10.1128/jcm.33.12.3091-3095.1995

Saito, T. B., Cunha-Filho, N. A., Pacheco, R. C., Ferreira, F., Pappen, F. G., Farias, N. A. R, Larsson C.E., & Labruna, M. B. (2008). Canine infection by rickettsiae and ehrlichiae in southern Brazil. *The American Journal of Tropical Medicine and Hygiene*, 79(1), 102-108. https://doi.org/10.4269/ajtmh.2008.79.102

Schouls, L. M., Van De Pol, I., Rijpkema, S. G., & Schot, C. S. (1999). Detection and identification of Ehrlichia, Borrelia burgdorferi sensu lato, and Bartonella species in Dutch Ixodes ricinus ticks. *Journal of Cinical Microbiology*, 37(7), 2215-2222. https://doi.org/10.1128/JCM.37.7.2215-2222.1999.

Senneville, E., Ajana, F., Lecocq, P., Chidiac, C., & Mouton, Y. (1991). Rickettsia conorii isolated from ticks introduced to northern France by a dog. *Lancet (British edition)*, 337(8742), 676. http://dx.doi.org/10.1016/0140-6736(91)92493-l

Smetanova, K., Schwarzova, K., & Kocianova, E. (2006). Detection of Anaplasma phagocytophilum, Coxiella burnetii, Rickettsia spp., and Borrelia burgdorferi sl in Ticks, and Wild-Living Animals in Western and Middle Slovakia. *Annals of the New York Academy of Sciences*, 1078(1), 312-315. https://doi.org/10.1196/annals.1374.058

Stanek, G., Wormser, G. P., Gray, J., & Strle, F. (2012). Lyme borreliosis. *The Lancet*, 379(9814), 461-473. https://doi.org/10.1016/S0140-6736(03)14798-8

Steere, A. (2000). Borrelia burgdorferi. *Lyme Disease, Lyme Borreliosis) in Mandell, Douglas and Bennetts' editors Principles and Practice of Infectious Diseases*, 7, 3071-3081. https://doi.org/10.1056/NEJM200107123450207

Talleklint, L., & Jaenson, T. G. (1998). Increasing geographical distribution and density of Ixodes ricinus (Acari: Ixodidae) in central and northern Sweden. *Journal of Medical Entomology*, 35(4), 521-526. https://doi.org/10.1093/jmedent/35.4.521

Vianna, M. C. B., Horta, M. C., Sangioni, L. A., Cortez, A., Soares, R. M., Mafra, C. L., Galvão, M. A. M., Labruna, M. B., & Gennari, S. M. (2008). Rickettsial spotted fever in capoeirao village, Itabira, Minas Gerais, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, 50, 297-301. https://doi.org/10.1590/s0036-46652008000500009

Weissenbock, H., & Holzmann, H. (1996). Tick-borne encephalitis in Austrian dogs. *The Veterinary Record*, 139(23), 575-576.

Wielinga, P. R., Gaasenbeek, C., Fonville, M., de Boer, A., de Vries, A., Dimmers, W., G. A. O. Jagers, L. M. Schouls, Borgsteede, F., & van der Giessen, J. W. (2006). Longitudinal analysis of tick densities and Borrelia, Anaplasma, and Ehrlichia infections of Ixodes ricinus ticks in different habitat areas in The Netherlands. *Applied and Environmental Microbiology*, 72(12), 7594-7601. https://doi.org/10.1128/AEM.01851-06