Vol.3, No.9, 501-503 (2013)
http://dx.doi.org/10.4236/ojpm.2013.39067

Open Journal of Preventive Medicine

Preliminary study of natural reservoirs as sentinels of *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* in Soria, northern Spain

Lourdes Lledó[1*], Consuelo Giménez-Pardo[1], José Luis Serrano[2]

[1]Departamento de Biomedicina y Biotecnología, Universidad Alcalá, Alcalá de Henares, Spain;
[*]Corresponding Author: lourdes.lledo@uah.es
[2]Consejería de Sanidad y Bienestar Social de la Junta de Castilla y León, Soria, Spain

Received 29 September 2013; revised 30 October 2013; accepted 9 November 2013

ABSTRACT

The serum of foxes and red deer from the Province of Soria (northern Spain) was screened in indirect immunofluorescence assays to determine whether these animals could be used as sentinels of the tick-borne pathogens *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. The results suggest that foxes and red deer would not make good sentinels in epidemiological studies on *E. chaffeensis* in this region, although red deer could be used as such for the study of *A. phagocytophilum* transmission.

Keywords: *Anaplasma phagocytophilum*; *Ehrlichia chaffeensis*; Foxes; Red Deer; Sentinels; Spain

1. INTRODUCTION

Ehrlichia chaffeensis and *Anaplasma phagocytophilum* are the causal agents of severe emerging and re-emerging human diseases [1]. Both are transmitted through the bite of an infected tick. In Europe, the primary vector is *Ixodes ricinus*, for which red deer (*Cervus elaphus*), foxes (*Vulpes vulpes*), cattle, sheep, goats and horses act as reservoirs [2]. In Spain, little is known about the epidemiology of these diseases. The present work reports a preliminary study, undertaken in the Province of Soria, to record the prevalence of the above pathogens in foxes and red deer, and to determine the value of these animals as sentinel species.

2. MATERIAL AND METHODS

2.1. Study Area

The Province of Soria is located in northern Spain (central point 41˚25'0"N, 2˚28'0"W; altitude range 1100 - 1650 m). It has a continental climate with cold winters and mild summers. The region has extensive forested areas and high shrubland [3].

2.2. Collection of Serum Samples

All the serum samples used in this work—30 from wild red deer and 30 from wild foxes—came from our group's frozen (−20˚C) serum collection.

2.3. Serological Assays

Indirect immunofluorescence assays (IFA) were performed to detect antibodies to *A. phagocytophilum* and *E. chaffeensis* in the tested serum samples according to Santos *et al*. [4] and Brouqui *et al*. [5]. Samples were diluted 1:40 in PBS and incubated on slides prepared with HL-60 cells infected with A. phagocytophilum (prepared with Arkansas strain) or DH82 cells infected with *E. chaffeensis* (prepared with Webster strain) (both types of slides were kindly supplied by R. Sousa and A.S. Santos of the Centro de Estudos de Vectores e Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal). The fluorescein-labelled conjugate was adapted according to the animal species studied (Sigma, St Louis, MO). Results were interpreted as positive when IgG titres of ≥40 were recorded. All positive samples were serially diluted to determine the endpoint titre, which was expressed as the reciprocal of the serum dilution.

2.4. Tick Counts

The number of different ticks on each animal from which serum was extracted was recorded in a database at the time of serum preparation. Ticks were enumerated

following the method of Dominguez-Peñafiel *et al*. [6].

3. RESULTS AND DISCUSSION

None of the fox serum samples examined was positive for *E. chaffeensis*, while three were positive for *A. phagocytophilum*. All the red deer serum samples were positive for *A. phagocytophilum* but negative for *E. chaffeensis.*

A total of 109 ticks were obtained from the red deer: 37 males (33.94%), 56 females (51.37%) and 11 nymphs (10.09%). The most common species identified was *Ixodes ricinus*, with 35 males (41.17%), 50 females (58.82%), followed by *Haemaphysalis punctata* with 1 male (20%) and 4 females (80%), and *Rhipicephalus bursa* with 1 male (33.33%) and 2 females (66.66%). As expected, most of the ticks found were adults (93 [85.32%]). The other work in the study area reported the same tick species on the same hosts [3].

Some 20% of the foxes were parasitised by ticks. The most common species identified (always as adults) were *Rhipicephalus sanguineus* (50%), *Ixodes canisuga* (16.6%), *Ixodes ricinus* (16.6%) and *Ixodes hexagonus* (16.6%). These species are similar to those recorded on foxes from Thuringia (Germany) [7], and the same as those recorded in foxes in eastern Spain [8] and the Spanish south [9]. *Dermacentor variabilis* and species of *Amblyoma* are known to act as vectors of *A. phagocytophilum* and Ehrlichia spp., but whether other ticks do so is unknown. The present results for the red deer suggest studies are needed to identify the role of other tick species in *A. phagocytophilum* transmission.

E. chaffeensis is the causal agent of human monocytotropic ehrlichiosis, and foxes are potential reservoirs for this bacterium [10]. *A. phagocytophilum* is the causal agent of monocytic anaplasmosis, and this pathogen has been confirmed in red deer in Slovakia [11], as well as in sheep, ticks and foxes in Hungary [12]. In Spain, it has been detected serologically and by PCR (in both the north and south) in ticks, roe deer, and cattle [13,14].

Since *Ehrlichia* and *Anaplasma* species can cause human disease [15-18], the prevalence of these pathogens in the study area, where contact between wild animals and people through outdoor activities and work, should be monitored. The present results suggest that foxes and red deer would not make good sentinels in epidemiological studies on *E. chaffeensis* in the study region, although red deer could be used as such for the study of *A. phagocytophilum* transmission. Red deer would seem to be involved in the natural cycle of this pathogen in this province.

4. ACKNOWLEDGEMENTS

We thank Drs. Rita Sousa and Ana Sofia Santos (Centro de Estudos de Vectores e Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal) for providing the antigen slides for IFA.

REFERENCES

[1] Blanco, J.R., Jado, I., Marín, M., Sanfeliu, I., Portillo. A., Anda. P., Pons. I. and Oteo, J.A. (2008) Diagnóstico microbiológico de las infecciones por patógenos bacterianos emergentes: Anaplasma, Bartonella, Rickettsia, Tropheryma whipplei. *Enfermedades Infecciosas y Microbiología Clínica*, **26**, 573-580. http://dx.doi.org/10.1157/13128275

[2] Kalinová, Z., Cisláková, L. and Halánová, M. (2009) Ehrlichiosis/Anaplasmosis. *Klinická Mikrobiologie a Infekcni Lekarstvi*, **15**, 210-213.

[3] Merino, F.J., Nebreda, T., Serrano, J.L., Fernández-Soto, P., Encinas, A. and Pérez-Sánchez, R. (2005) Tick species and tick-borne infections identified in population from a rural area of Spain. *Epidemiology and Infection*, **133**, 943-949. http://dx.doi.org/10.1017/S0950268805004061

[4] Santos, A. S., Alexandre, N., Sousa, R., Núncio, M. S., Bacellar, F.J. and Dumler, S. (2009) Serological and molecular survey of Anaplasma species infection in dogs with suspected tickborne disease in Portugal. *Veterinary Record*, **164**, 168-171. http://dx.doi.org/10.1136/vr.164.6.168

[5] Brouqui, P., Lecam, C., Olson, J. and Raoult, D. (1994) Serologic diagnosis of human monocytic ehrlichiosis by immunoblot analysis. *Clinical and Diagnostic Laboratory Immunology*, **1**, 645-649.

[6] Domínguez, G. (2004) North Spain (Burgos) wild mammals ectoparasites. *Parasite*, **11**, 262-272.

[7] Meyer-Kayser, E., Hoffmann, L., Silaghi, C., Pfister, K., Mahling, M. and Passos, L.M. (2012) Dynamics of tick infestations in foxes in Thuringia, Germany. *Ticks and Tick-Borne Diseases*, **3**, 232-239. http://dx.doi.org/10.1016/j.ttbdis.2012.05.004

[8] Estrada-Peña, A., Osácar, J.J., Gortázar, C., Calvete, C. and Lucientes, J. (1992) An account of the ticks of the northeastern of Spain (Acarina: Ixodidae). *Annales de Parasitologie Humaine et Comparée*, **67**, 42-49.

[9] Millán, J., Ruiz-Fons, F., Márquez, F.J., Viota, M., López-Bao, J.V. and Paz Martín-Mateo, M. (2007) Ectoparasites of the endangered Iberian lynx Lynx pardinus and sympatric wild and domestic carnivores in Spain. *Medical and Veterinary Entomology*, **21**, 248-254. http://dx.doi.org/10.1111/j.1365-2915.2007.00696.x

[10] Davidson, W.R., Lockhart, J.M., Stallknecht, D.E. and Howerth, E.W. (1999) Susceptibility of red and gray foxes to infection by Ehrlichia chaffeensis. *Journal of Wildlife Diseases*, **35**, 696-702. http://dx.doi.org/10.7589/0090-3558-35.4.696

[11] Víchová, B., Majláthová, V., Nováková, M., Stanko, M., Hviščová, I., Pangrácová, L., Chrudimský, T., Curlík, J. and Peťko, B. (2013) Anaplasma infections in ticks and reservoir host from Slovakia. *Infection, Genetics and Evolution*, in Press.

[12] Sréter, T., Kálmán, D., Sréterné Lancz, Z., Széll, Z. and Egyed, L. (2005) Babesia microti and Anaplasma phago-

cytophilum: Two emerging zoonotic pathogens in Europe and Hungary. *Orvosi Hetilap*, **46**, 595-600.

[13] de la Fuente, J., Ruiz-Fons, F., Naranjo, V., Torina, A., Rodríguez, O. and Gortázar, C. (2008) Evidence of Anaplasma infections in European roe deer (Capreolus capreolus) from southern Spain. *Research in Veterinary Science*, **84**, 382-386. http://dx.doi.org/10.1016/j.rvsc.2007.05.018

[14] Portillo, A., Pérez-Martínez, L., Santibáñez, S., Santibáñez, P., Palomar, A.M. and Oteo, J.A. (2011) Anaplasma spp. in wild mammals and Ixodes ricinus from the north of Spain. *Vector-Borne and Zoonotic Diseases*, **11**, 3-8. http://dx.doi.org/10.1089/vbz.2009.0214

[15] Ganguly, S. and Mukhopadhayay, S.K. (2008) Tick-borne ehrlichiosis infection in human beings. *Journal of Vector Borne Diseases*, **45**, 273-280.

[16] Schneider, J.G. (2009) Human ehrlichiosis: A case study. *Clinical Laboratory Science*, **22**, 3-8.

[17] Cochez, C., Ducoffre, G., Vandenvelde, C., Luyasu, V. and Heyman, P. (2011) Human anaplasmosis in Belgium: A 10-year seroepidemiological study. *Ticks and Tick-Borne Diseases*, **2**, 156-159. http://dx.doi.org/10.1016/j.ttbdis.2011.06.004

[18] Hao, Q., Geng, Z., Hou, X.X., Tian, Z., Yang, X.J., Jiang, W.J., Shi, Y., Zhan, Z.F., Li, G.H., Yu de, S., Wang, H.Y., Xu, J.G. and Wan, K.L. (2013) Seroepidemiological investigation of Lyme disease and human granulocytic anaplasmosis among people living in forest areas of eight provinces in China. *Biomedical and Environmental Sciences*, **26**, 185-189.

