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TICK-BORNE BACTERIA IN MOUFLON AND THEIR ECTOPARASITES IN CYPRUS

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1 2 ABSTRACT: The Cypriot mouflon (*Ovis orientalis ophion*), a once almost extirpated species of wild sheep, is under strict surveillance because it can be threatened by likely transmission of pathogenic bacteria, such as *Anaplasma* spp., *Rickettsia* spp., and *Coxiella burnetii*, primarily from domestic ungulates. We collected 77 blood samples from Cypriot mouflon and 663 of their ectoparasites (*Rhipicephalus turanicus, Rhipicephalus sanguineus, Rhipicephalus bursa, Hyalomma anatolicum excavatum, Hyalomma marginatum, Haemaphysalis punctata, Haemaphysalis sulcata,* and *Ixodes gibossus*) and tested them by polymerase chain reaction and sequencing. Twenty-three mouflon blood samples (30%) were positive for *C. burnetii*, 23 (30%) for *Rickettsia* spp., and 8 (10%) for *Anaplasma ovis.* Of 109 pools of ectoparasites, 32.1% were positive for *C. burnetii*, 28.4% for *Rickettsia* spp., and 10.9% for *A. ovis;* 11.9% were positive for both *C. burnetii* and *Rickettsia* spp., 6.4% for both *Rickettsia* spp. and *A. ovis*, and 2.8% for all three pathogens. This is the first survey that records the presence of tick-borne pathogens, both in the Cypriot mouflon and in ticks parasitizing it.
Key words: *Anaplasma*, *Coxiella burnetii*, Cyprus, mouflon, *Rickettsia*.

INTRODUCTION

The Cypriot mouflon (*Ovis orientalis ophion*) or Agrino is a subspecies of wild sheep endemic to Cyprus. It is the largest terrestrial wild mammal in Cyprus and is of Asiatic origin (Payne, 1968; Vigne, 1988). Its presence on Cyprus can be traced back 10,000 yr. It is the smallest species of wild sheep that inhabits the forest of Pafos, a mountainous landscape of 620,000 ha in the northwestern Troodos range. Along with the mouflon of Sardinia and Corsica (*Ovis gmelini mussimon*) it is the only forest dweller among the *Caprinae* (Payne, 1968; Hemmer, 1990). The population in Cyprus, estimated to be 3,000 animals (Kassinis and Papageorgiou, 2000), is strictly protected under Cypriot legislation and has been included in Annex II/IV of 92/43 EU Habitats Directive as a European priority species. Higher mouflon population densities occur in the forest periphery where vegetation is lower and more variable and pastures are more extensive. In some parts of the forest periphery goat and sheep herding is intensive, especially north and southwest of the forest where domestic animals and mouflon share pasture (Kassinis, unpubl.). The species' health is closely monitored by the Game Fund Service in collaboration with the Department of Cyprus Veterinary Services because there is a high probability of infection by pathogenic bacteria carried by domestic ungulates. Functions of the Cyprus Game Fund Service include wildlife law enforcement and management of all game and protected mammalian and avian species including habitat improvement. Other countries have established genetic management and reproductive biotechnologies (Ptak et al., 2002) in an attempt to preserve the wild European mouflon. Our aim was to investigate the presence *Anaplasma* spp., *Rickettsia* spp., and *Coxiella burnetii* in Cypriot mouflon and their ectoparasites.

MATERIALS AND METHODS

Sampling

Blood samples from 77 mouflon from the Pafos forest were examined between November 2002 and December 2006. Dead and sick

animals were transferred to the Cyprus Veterinary Services along with blood samples taken from live-trapped and released animals during Game Fund's annual trapping program. Blood samples were collected using filter papers, which were then stored in vials at −20 C until further treatment. Ectoparasites, whenever found, were collected and stored at −20 C. Sex, age, and global positioning system coordinates of each animal location were recorded.

Sample preparation

Samples were processed at the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses, and Geographical Medicine (WHO Collaborating Center) in Crete, Greece. Filter papers were cut into 1×1-cm squares and stored separately for each animal. Blood was eluted with 490 µl phosphate buffered saline (PBS) at pH 7.2 with 10 µl fetal bovine serum held overnight at 4 C. Two hundred microliters of the solution were transferred to new tubes for DNA extraction and stored at −20 C. Ectoparasites were separated according to species and sex based on taxonomic keys (Gil-Collado, 1961; Gil-Collado et al., 1979). They were then disinfected using 70% ethanol and cut into small pieces in 200 µl PBS. Pools (of 200 µl final volume) were created based on ectoparasite species and animal origin. Each pool contained a maximum of 10 ticks; the polymerase chain reaction (PCR) method was designed to have a sensitivity of detection of one positive sample per pool of 10 samples. All samples were stored at −20 C.

Analysis of samples

DNA extractions were carried out using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. PCR amplification was carried out for detection of *C. burnetii*, *Rickettsia* spp., and *Anaplasma* spp. in blood samples and ectoparasites. For detection of DNA of *Rickettsia* spp., primers RpCS 877p and RpCS 1258n, which amplify a 381 base pair (bp) region of the citrate synthase *gltA* gene (Roux et al., 1997), along with the primer set Rr19070p and Rr190602n, which amplify the *ompA* outer membrane protein gene giving a product of 532 bp (Regnery et al., 1991), were used. For *C. burnetii* DNA, primers CB1 and CB2, which amplify a 257 bp region of the superoxide dismutase gene (Spyridaki et al., 1998), were used. For *Anaplasma* spp., primers EHR16SD and EHR16SR, which amplify a 345 bp product of the 16s rRNA gene (Brouqui et al., 2001), primers HSPB and HSPC to amplify an approximately 1650 bp fragment of the *groEL* gene (Lew et al., 2003), and primers msp43 and msp45 to amplify an approximately 850 bp fragment of the *msp4* gene (de la Fuente et al., 2007) were used. Negative controls (distilled water) were used every 10 samples. The first sample found positive by PCR, and sequence analysis was used as the positive control. PCR products were electrophoresed on 1–2% agarose gel and visualized following 30-min incubation in GelStar® Nucleic Acid Gel Stain (Lonza Rockland, Rockland, Maine, USA) at room temperature according to the manufacturer's instructions.

PCR products were purified (PCR product purification kit; Qiagen) and directly sequenced at a CEQ 8000 Beckman Coulter Sequencer (Bioanalytica-Genotype, Athens, Greece). All PCR products were sequenced twice to verify sequences. Nucleic acid sequences were processed using nucleotide BLASTn, Chromas v1.49, and Lasergene Version 7.1 software. Sequences were submitted to GenBank.

RESULTS

Parasitism

Of the 77 mouflon, 62 (81%) were parasitized at the time of examination, and 28 (36%) suffered from infestation by two, three, or four tick species. Of 663 ectoparasites collected, 268 (40.4%) were species of *Rhipicephalus* (6.9% *R. turanicus*, 0.9% *R. sanguineus*, 23.6% *R. bursa*), 84 (12.7%) were *Hyalomma* species (12.5% *H. anatolicum excavatum*, 0.15% *H. marginatum*), 291 (43.9%) were *Haemaphysalis* (2.3% *H. punctata*, 41.6% *H. sulcata*,), and 23 (3.5%) were *Ixodes gibossus*. The majority of ectoparasites (55.5%) were males; very few (5.5%) were nymphal (sex not determined; Fig. 1). The largest numbers of ticks were collected during summer and autumn; *R. bursa* was most common in summer, and *H. sulcata* was most common in autumn. Infestation rates were lower during winter and spring; *H. sulcata* was the most abundant species during winter but was almost absent during spring and summer.

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PCR and sequencing

Mouflon: Of the 77 mouflon examined, 23 (30%) were PCR positive for *C. burnetii*

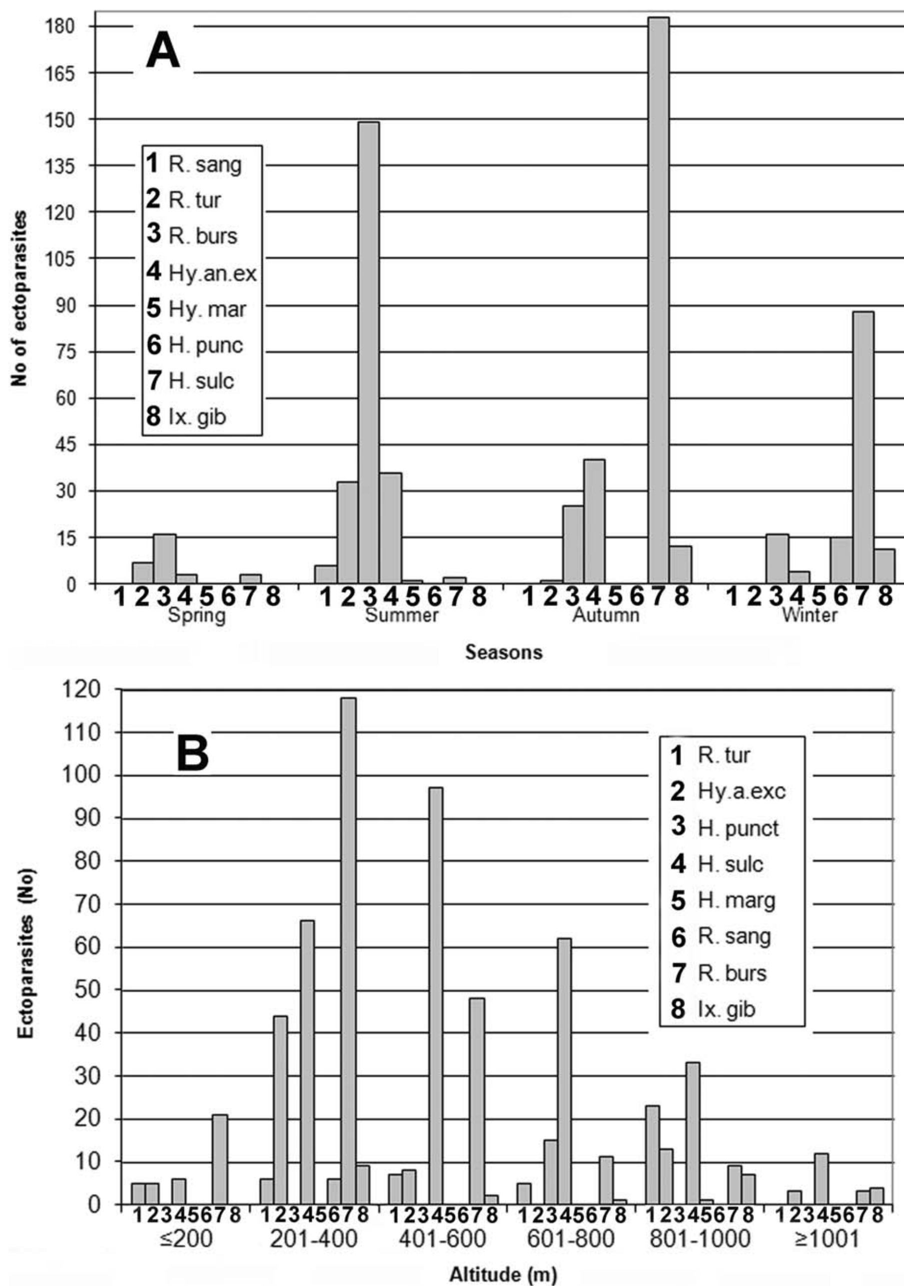

FIGURE 1. Seasonal (A) and elevational (B) distribution of ticks collected from mouflon in the forest of Pafos, Cyprus, November 2002–December 2007. Numbers correspond to tick species (*R. tu* = *Rhipicephalus turanicus*; *Hy.a.ex* = *Hyalomma anatolicum excavatum*; *H. punct* = *Haemaphysalis punctata*; *H. sulc* = *Haemaphysalis sulcata*; *H. marg* = *Hyalomma marginatum*; *R. sang* = *Rhipicephalus sanguineus*; *R. bur* = *Rhipicephalus bursa*; *Ix. gib* = *Ixodes gibossus*).

(GenBank accession number EU448143), 23 (30%) for *Rickettsia* spp. (EU448154, EU448155, EU448161), and 8 (10%) for *A. ovis* (*msp4* accession number GQ261180, *groEL* accession number GQ261181). Twelve (16%) animals were PCR positive for both *C. burnetii* and *Rickettsia* spp. The strains identified in

TABLE 1. Cypriot mouflon (*Ovis orientalis ophion*) and their ectoparasite species tested by PCR for *Coxiella burnetii*, *Rickettsia* spp., and *Anaplasma ovis.* Accession number EU448155 corresponds to *R. rhipicephali*, EU448154 to *R. endosymbiont,* and EU448161 to *R. massiliae.*

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Ectoparasite species[a]	Total tested	*Coxiella burnetii*, no. positive (%)	GenBank accession no.	*Rickettsia* spp., no. positive (%)	GenBank accession no.	*Anaplasma ovis*, no. positive (%)	GenBank accession no.
Ectoparasites (pools)							
Total tested	109	35 (32.1)		31 (28.4)		12 (10.9)	
R. tur	10	4 (40)	EU448145	2 (20)	EU448155	0 (0)	
Hy.a.ex	15	2 (13)		0 (0)		0 (0)	
H. punct	2	2 (100)		2 (100)	EU448154	0 (0)	
H. sulc	41	5 (12)		23 (56)		10 (24)	GQ261180, GQ261181
H. marg	1	0 (0)		0 (0)		0 (0)	
R. sang	2	2 (100)		0 (0)		0 (0)	
R. bur	35	14 (40)	EU448144	8 (23)	EU448161	0 (0)	
Ix. gib	3	0 (0)		0 (0)		0 (0)	
Mouflons (no.)	77	23 (30)	EU448143	23 (30)		8 (10.4)	GQ261180, GQ261181

[a] *R. tur* = *Rhipicephalus turanicus*; *Hy.a.ex* = *Hyalomma anatolicum excavatum*; *H. punct* = *Haemaphysalis punctata*; *H. sulc* = *Haemaphysalis sulcata*; *H. marg* = *Hyalomma marginatum*; *R. sang* = *Rhipicephalus sanguineus*; *R. bur* = *Rhipicephalus bursa*; *Ix. gib* = *Ixodes gibossus*.

ticks were identical to the sequences retrieved from mouflon (Table 1).

Ectoparasites: Of the 109 pools of ectoparasites, 35 (32.1%) were PCR positive for *C. burnetii*, 31 (28.4%) for *Rickettsia* spp., and 12 (10.9%) for *A. ovis;* 13 (11.9%) were positive both for *C. burnetii* and *Rickettsia* spp., 7 (6.4%) for *Rickettsia* spp. and *A. ovis*, and 3 (2.8%) for all three pathogens. *Coxiella burnetii* was detected in *R. turanicus* (EU448145), *R. sanguineus, R. bursa* (EU448144), *H. a. excavatum, H. sulcata,* and *H. punctata*. *Rickettsia* sp. was detected in *R. turanicus* (*R. rhipicephali*, EU448155), *R. bursa* (*R. massiliae*, EU448161), *H. punctata* (*R.* endosymbiont, EU448154), and *H. sulcata*. *Anaplasma ovis* was detected in *H. sulcata* only (*msp4* accession number GQ261180; *groEL* accession number GQ261181; Table 1). All sequences retrieved from both mouflon and ectoparasites were identical to the sequences obtained from blood samples of animals of veterinary importance (goats, sheep) and their ectoparasites, which were collected from the area of Pafos forest (Psaroulaki et al., unpubl.).

DISCUSSION

The endemic Cypriot mouflon is a strictly protected species under National and European Union legislation and listed as endangered under the International Union for the Conservation of Nature 1996 Red list (Shackleton and Lovari, 1997). One of the most serious conservation problems that the species faces is the danger of infection from pathogens such as *Rickettsia* spp., *A. ovis,* and *C. burnetii*, primarily due to sharing pastures with domestic ungulates in the forest periphery.

The presence of *H. m. marginatum, R. bursa,* and *Dermacentor marginatus* on European wild boar and Iberian red deer has been recorded (de la Fuente et al., 2004; Ruiz-Fons et al., 2006). These three species are representatives of the tick fauna commonly found on Mediterranean ungulates. In a study of the Iberian mouflon, Alonso et al. (2004) found 95.8% of the tested mouflon parasitized by *R. bursa, Boophilus annulatus*, *R. sanguineus*, *R. turanicus*, *H. sulcata*, *Ixodes ricinus ricinus*, *H. punctata,* and *D. marginatus* with two, three, or four tick

species found on 47.8% of the mouflon in that study. Only 36% (28 of 77 mouflon) in our study were infested by multiple tick species. *Dermacentor marginatus* and *I. ricinus*, both mentioned by de la Fuente et al. (2004) and Alonso et al. (2004), may not be common in Cyprus.

Emerging tick-borne diseases constitute an increasing threat for human and animal health worldwide and notably in Europe (Parola, 2004). Characterization of the prevalence of pathogens in tick species that parasitize wildlife reservoirs may provide important information for the assessment of risk of emerging tick-borne disease outbreaks. This is the first report demonstrating the presence of *C. burnetti*, *Rickettsia* spp., and *A. ovis* in the Cypriot mouflon and ticks infesting it. Although Hubalek et al. (1993) found Sardenian mouflon positive for *C. burnetii* using immunofluorescent antibody tests, to our knowledge this is the first report identifying *Rickettsia* spp. and *A. ovis* in mouflon. Sixteen percent of the mouflon we tested were infected by both *Rickettsia* spp. and *C. burnetii*. Since mouflon may come into close contact with domestic animals, coinfection of ticks with several of these organisms may increase the severity of disease in cattle (Hofle et al., 2004; Hofmann-Lehmann et al., 2004) and humans (Krause et al., 1996).

We demonstrated *A. ovis* in *H. sulcata* ticks only. *Anaplasma phagocytophilum* was reported for the first time in European ticks in 1997 (von Stedingk et al., 1997). De la Fuente et al. (2004) identified *A. phag*ocytophilum and *A. marg*inale in *H. m. marginatum* and *R. bursa* ticks and proposed a role of these tick species in the transmission of tick-borne zoonoses in wildlife and domestic animals. Loftis et al. (2006) also detected *A. marginale* in *H. a. excavatum* from cattle.

Contrary to Punda-Polic et al. (2002) and Georges et al. (2001), we were able to detect *Rickettsia* spp. in *R. turanicus, R. bursa, H. punctate,* and *H. sulcata* ticks. Punda-Polic et al. (2002) sequenced a portion of the *ompA* and *ompB* genes related to *Rickettsia aeschlimannii* in *H. marginatum* ticks, as did Fernandez-Soto et al. (2003). The latter author also detected *Rickettsia* in *H. punctata, R. bursa*, *R. turanicus,* and *R. sanguineus* ticks. Bernasconi et al. (2002) found *R. sanguineus* ticks infected with a *Rickettsia* identified as *R. massiliae*. Although we failed to detect *Rickettsia spp.* in *H. a. excavatum* ticks, Psaroulaki et al. (2005) found *R. mongolotimonae* both in humans and in *H. a. excavatum*.

Our finding of *C. burnetii* in *R. sanguineus, R. turanicus. R. bursa, H. a. excavatum, H. sulcata,* and *H. punctata* ticks is in accordance with findings of Bernasconi et al. (2002), who also detected *C. burnetii* in *R. sanguineus* and *R. turanicus*. Loftis et al. (2006) also detected *C. burnetii* in *H. a. excavatum* removed from cattle. Although we failed to detect *C. burnetii* in *H. marginatum* ticks, Psaroulaki et al. (2006) reported its presence in this tick species. Rehacek et al. (1970) isolated *C. burnetii* from *H. punctata* ticks in Slovakia; this finding is further supported by our research. To our knowledge, ours is the first report demonstrating *C. burnetii* in *H. sulcata, R. bursa,* and *I. gibbosus* ticks. Psaroulaki et al. (2006) collected these tick species (*H. sulcata* from goats, *R. bursa* and *I. gibbosus* from goats, sheep, cattle, and horses) on the Greek island of Cephalonia but failed to obtain PCR evidence of the pathogen.

The identity among the strains from mouflon and animals of veterinary importance in the region implies the interchange of these pathogens between wild and domesticated animals. Our results underscore the necessity to strongly consider appropriate action to protect the endangered Cypriot mouflon from potential disease threats associated with tick-borne pathogens of domesticated ungulates.

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LITERATURE CITED

Alonso, F. D., J. Gomis, M. R. Ruiz de Ybanez, A. Gutierrez, J. Sierra, and C. Martinez-Carrasco. 2004. Tick infestation on wild ruminants in the Sierra de Cazorla, Segura y las Villas Natural Park, Spain. *In* Proceedings of the XII Congress of the Mediterranean Federation for Health and Production of Ruminants, 16–19 September 2004, Istanbul, Turkey, pp. 000–000.

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Bernasconi, M. V., S. Casati, O. Peter, and J. C. Piffaretti. 2002. *Rhipicephalus* ticks infected with *Rickettsia* and *Coxiella* in Southern Switzerland (Canton Ticino). Infection Genetics and Evolution 2: 111–120.

Brouqui, P., P. E. Fournier, and D. Raoult. 2001. Doxycycline and eradication of microfilaremia in patients with loiasis. Emerging Infectious Diseases 7: 604–605.

de la Fuente, J., J. Vicente, U. Hofle, F. Ruiz-Fons, I. G. Fernandez de Mera, R. A. van den Bussche, K. M. Kocan, and C. Gortazar. 2004. Anaplasma infection in free-ranging Iberian red deer in the region of Castilla–La Mancha, Spain. Veterinary Microbiology 100: 163–173.

———, M. W. Atkinson, V. Naranjo, I. G. Fernandez de Mera, A. J. Mangold, K. A. Keating, and K. M. Kocan. 2007. Sequence analysis of the msp4 gene of *Anaplasma ovis* strains. Veterinary Microbiology 119: 375–381.

Fernandez-Soto, P., A. Encinas-Grandes, and R. Perez-Sanchez. 2003. *Rickettsia aeschlimannii* in Spain: Molecular evidence in *Hyalomma marginatum* and five other tick species that feed on humans. Emerging Infectious Diseases 9: 889–890.

Georges, K., G. R. Loria, S. Riili, A. Greco, S. Caracappa, F. Jongejan, and O. Sparagano. 2001. Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Veterinary Parasitology 99: 273–286.

Gil-Collado, J. 1961. Insectos y ácaros de los animales domésticos. Ed. Salvat, Barcelona, Spain, 591 pp.

———, J. L. Guillen Llera, and L. M. Zapatero Ramos. 1979. Claves para la identificación de los ixodoidea españoles (adultos). Revista Ibérica de Parasitología 39: 107–118.

Hemmer, H. 1990. Domestication: The decline of environmental appreciation. Cambridge University Press, Cambridge, England, 208 pp.

Hofle, U., J. Vicente, D. Nagore, A. Hurtado, A. Pena, J. de la Fuente, , and C. Gortazar. 2004. The risks of translocating wildlife: Pathogenic infection with *Theileria* sp. and *Elaeophora elaphi* in an imported red deer. Veterinary Parasitology 126: 387–395.

Hofmann-Lehmann, R., M. L. Meli, U. M. Dreher, E. Gonczi, P. Deplazes, U. Braun, M. Engels, J. Schupbach, K. Jorger, R. Thoma, C. Griot, K. D. Stark, B. Willi, J. Schmidt, K. M. Kocan, and H. Lutz. 2004. Concurrent infections with vector-borne pathogens associated with fatal hemolytic anemia in a cattle herd in Switzerland. Journal of Clinical Microbiology 42: 3775–3780.

Hubalek, Z., Z. Juricova, S. Svobodova, and J. Halouzka. 1993. A serologic survey for some bacterial and viral zoonoses in game animals in the Czech Republic. Journal of Wildlife Diseases 29: 604–607.

Kassinis, N. I., and K. Papageorgiou. 2000. The endemic Cyprus mouflon: Population status and conservation. Game Fund report, Cyprus Ministry of Interior, 8 pp.

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Krause, P. J., S. R. Telford III, A. Spielman, V. Sikand, R. Ryan, D. Christianson, G. Burke, P. Brassard, R. Pollack, J. Peck, and D. H. Persing. 1996. Concurrent Lyme disease and babesiosis: Evidence for increased severity and duration of illness. Journal of the American Veterinary Medical Association 275: 1657–1660.

Lew, A. E., K. R. Gale, C. M. Minchin, V. Shkap, and D. T. De Waal. 2003. Phylogenetic analysis of the erythrocytic *Anaplasma* species based on 16S rDNA and GroEL (HSP60) sequences of *A. marginale*, *A. centrale*, and *A. ovis* and the specific detection of *A. centrale* vaccine strain. Veterinary Microbiology 92: 145–160.

Loftis, A. D., W. K. Reeves, D. E. Szumlas, M. M. Abbassy, I. M. Helmy, J. R. Moriarity, and G. A. Dasch. 2006. Rickettsial agents in Egyptian ticks collected from domestic animals. Experimental and Applied Acarology 40: 67–81.

Parola, P. 2004. Tick-borne rickettsial diseases: Emerging risks in Europe. Comparative Immunology, Microbiology & Infectious Diseases 27: 297–304.

Payne, S. 1968. The origin of domestic sheep and goats: A reconsideration in the light of the fossil evidence. Proceedings of the Prehistoric Society 34: 368–384.

Psaroulaki, A., A. Germanakis, A. Gikas, E. Scoulica, and Y. Tselentis. 2005. Simultaneous detection of "*Rickettsia mongolotimonae*" in a patient and in a tick in Greece. Journal of Clinical Microbiology 43: 3558–3559.

———, D. Ragiadakou, G. Kouris, B. Papadopoulos, B. Chaniotis, and Y. Tselentis. 2006. Ticks, tick-borne rickettsiae, and *Coxiella burnetii* in the Greek Island of Cephalonia. Annals of the New York Academy Sciences 1078: 389–399.

Ptak, G., M. Clinton, B. Barboni, M. Muzzeddu, P. Cappai, M. Tischner, and P. Loi. 2002. Preservation of the wild European mouflon: The first example of genetic management using a complete program of reproductive biotechnologies. Biology of Reproduction 66: 796–801.

Punda-Polic, V., M. Petrovec, T. Trilar, D. Duh,

N. Bradaric, Z. Klismanic, and T. Avsic-Zupanc. 2002. Detection and identification of spotted fever group Rickettsiae in ticks collected in southern Croatia. Experimental and Applied Acarology 28: 169–176.

Regnery, R., C. L. Spruill, and B. D. Plikaytis. 1991. Genotypic identification of Rickettsiae and estimation of intraspecies sequence divergence for portions of two Rickettsial genes. Journal of Bacteriology 173: 1576–1589.

Rehacek, J., R. Brezina, and M. Majersk. 1970. Investigation of Q fever in Slovakia I: Isolation of agent from ticks and serological surveys in small mammals in Zvolen and Lueenec district of central Slovakia. Journal of Hygiene Epidemiology Microbiology and Immunology 14: 230–239.

Roux, V., E. Rydkina, M. Eremeeva, and D. Raoult. 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the Rickettsiae. International Journal of Systematic and Evolutionary Bacteriology 47: 252–261.

Ruiz-Fons, F., I. G. Fernandez de Mera, P. Acevedo, U. Hofle, J. Vicente, J. de la Fuente, and C. Gortazar. 2006. Ixodid ticks parasitizing Iberian red deer (*Cervus elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: Geographical and temporal distribution. Veterinary Parasitology 140: 133–142.

Shackleton, D. M., and S. Lovari. 1997. Conservation of Eurasian wild sheep: An overview. *In* Proceedings of the Second International Symposium on Mediterranean Mouflon, Cyprus, pp. 20–36.

Spyridaki, I., A. Gikas, D. Kofteridis, A. Psaroulaki, and Y. Tselentis. 1998. Q fever in the Greek ssland of Crete: Detection, isolation, and molecular identification of eight strains of *Coxiella burnetii* from clinical samples. Journal of Clinical Microbiology 36: 2063–2067.

Vigne, J. D. 1988. Les Mammifères post-glaciaires de Corse. Étude Archéozoologique (26e supplement a Gallia Préhistoire). Éditions du Centre National de la Recherche Scientifique, Paris, France, 337 pp.

Von Stedingk, L. V., M. Gurtelschmid, H. S. Hanson, R. Gustafson, L. Dotevall, E. O. Engvall, and M. Granstrom. 1997. The human granulocytic ehrlichiosis (HGE) agent in Swedish ticks. Clinical Microbiology and Infection 3: 573–574.

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