

Vector-Borne and
Zoonotic Diseases

Vector-Borne and Zoonotic Diseases: <http://mc.manuscriptcentral.com/vbz>

Molecular detection of Hemoprotozoa and Rickettsia sp in arthropods collected from wild animals in the Burgos province, Spain

Journal:	<i>Vector-Borne and Zoonotic Diseases</i>
Manuscript ID:	VBZ-2009-0114.R3
Manuscript Type:	Original Research
Date Submitted by the Author:	08-Oct-2009
Complete List of Authors:	Lledó, Lourdes; Alcalá de Henares University, Microbiology and Parasitology Consuelo, Gimenez; Alcalá de Henares University, Microbiology and Parasitology Dominguez, Gerardo; Consejería de Sanidad y Bienestar Social de la Junta de Castilla y León Sousa, Rita; Centro de Estudos de Vetores e Doenças Infecciosas, Instituto Nacional de Saúde Isabel, Gegundez; Alcalá de Henares University, Microbiology and Parasitology Nieves, Casado; Alcalá de Henares University, Microbiology and Parasitology Criado, Angel; Alcalá de Henares University, Microbiology and Parasitology
Keyword:	Babesia, Epidemiology, Hepatozoon, Rickettsia, vector-borne
Abstract:	Limited information on presence of bacterial and hematozoan infections in parasitic arthropods from Spain is available. In an attempt to address this issue, prevalence of Theileria, Babesia, Hepatozoon and Rickettsia species was investigated by PCR plus sequencing. In a survey for zoonotic pathogens in ectoparasites, 42 wild animals (which included rodents, carnivores, Sciuridae and Cervidae) were captured in Burgos (Spain). A total of 258 arthropods (including 107 ticks, 76 fleas and 73 mites) were collected from these mammals. Molecular diagnostic results showed that: i) Rickettsia felis was found in fleas (2 Ctenocephalides felis), ii) Hepatozoon sp infected some fleas (2 Ctenocephalides sp and a DNA pool of Ceratophyllus sciurorum) and Acari (1 Neotrombicula sp) and iii) Theileria annae was found in Ixodes ricinus and I. hexagonus (each a single infected specimen). All microorganisms and parasites were genetically identical to pathogens already described in Spain or elsewhere. Infected arthropods were recovered from beech marten, bank vole, squirrel, wood mouse and red fox. Our findings emphasize the potential risk

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

	for transmission of rickettsias to humans (namely <i>R. felis</i>) in Burgos, since <i>C. felis</i> is capable to seek out humans for feeding. No hemoprotozoa with proven significance as human pathogens were found in the survey. However, diagnostic of <i>Theileria annae</i> in ticks recovered from wild canids suggests possible connection links of sylvatic and domestic cycles for some piroplasmida.



For Peer Review

1
2
3
4 **Molecular detection of Hemoprotozoa and *Rickettsia* sp in arthropods collected from wild**
5
6 **animals in the Burgos province, Spain**
7

8
9 **L. LLEDÓ¹, C. GIMÉNEZ-PARDO¹, G. DOMÍNGUEZ-PEÑAFIEL², R. SOUSA³, MI.**
10
11 **GEGÚNDEZ¹, N. CASADO¹, A. CRIADO¹**
12

13
14 ¹Departamento de Microbiología y Parasitología, Universidad de Alcalá, Spain
15

16
17 ² Consejería de Sanidad y Bienestar Social de la Junta de Castilla y León, Spain
18

19
20 ³Centro de Estudos de Vetores e Doenças Infecciosas, Instituto Nacional de Saúde,
21 Portugal
22

23 **Corresponding author/correspondence address and reprint to:**
24

25 Lourdes Lledó MD, PhD
26

- 27
- 28 ▪ Departamento de Microbiología y Parasitología, Universidad de Alcalá,
 - 29
 - 30 ▪ Ctra. Madrid-Barcelona, Km. 33,6- 28871- Alcalá de Henares, Madrid (España)
 - 31
 - 32 ▪ phone: 34 91 8854794
 - 33
 - 34 ▪ fax: 34 91 8854663
 - 35
 - 36 ▪ e-mail: lourdes.lledo@uah.es
 - 37
 - 38
 - 39 ▪ **Subject categories:** Epidemiology
 - 40
 - 41
 - 42
 - 43
 - 44

45 **RUNNING TITLE:** Hemoprotozoa and *Rickettsiae* in arthropods
46

47 **KEY WORDS:** Epidemiology, *Hepatozoon*, *Babesia*, *Theileria*, *Rickettsiae*, Vector-borne.
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Limited information on presence of bacterial and hematozoan infections in parasitic arthropods from Spain is available. In an attempt to address this issue, prevalence of *Theileria*, *Babesia*, *Hepatozoon* and *Rickettsia* species was investigated by PCR plus sequencing. In a survey for zoonotic pathogens in ectoparasites, 42 wild animals (which included rodents, carnivores, Sciuridae and Cervidae) were captured in Burgos (Spain). A total of 258 arthropods (including 107 ticks, 76 fleas and 73 mites) were collected from these mammals. Molecular diagnostic results showed that: i) *Rickettsia felis* was found in fleas (2 *Ctenocephalides felis*), ii) *Hepatozoon* sp infected some fleas (2 *Ctenophthalmus* sp and a DNA pool of *Ceratophyllus sciurorum*) and Acari (1 *Neotrombicula* sp) and iii) *Theileria annae* was found in *Ixodes ricinus* and *I. hexagonus* (each a single infected specimen).

All microorganisms and parasites were genetically identical to pathogens already described in Spain or elsewhere. Infected arthropods were recovered from beech marten, bank vole, squirrel, wood mouse and red fox. Our findings emphasize the potential risk for transmission of rickettsias to humans (namely *R. felis*) in Burgos, since *C. felis* is capable to seek out humans for feeding. No hemoprotozoa with proven significance as human pathogens were found in the survey. However, diagnostic of *Theileria annae* in ticks recovered from wild canids suggests possible connection links of sylvatic and domestic cycles for some piroplasmida.

INTRODUCTION

During the past few years, there has been in Spain an increase of the incidence of some zoonoses, especially those transmitted by arthropod vectors (Blanco and Oteo 2006). Climate change and global warming are inducing some ecological changes in living conditions of animal reservoirs. This may lead to increased contact to humans, which in turn contributes to more disease cases. After mosquitoes, ticks are the most important vectors of pathogens that can cause disease to man. Although most of *Rickettsiae* are transmitted by ticks, other vectors such as fleas, lice and mites can also be important vectors of these bacteria. Fleas are vectors of *Rickettsia typhi*, and *R. felis*. *R. prowazekii* and *R. akari* are transmitted by lice and mites respectively. Emerging zoonoses caused by *Babesia* spp and *Theileria* spp are diseases that mainly affect domestic animals and to lesser extent humans. Both genera are transmitted by ixodid tick bites (Blaschitz *et al.* 2008). In other respects, *Hepatozoon* sp has a life cycle that includes two hosts: the invertebrate (definitive) host which is a tick, louse, flea, or mosquito, and the vertebrate (intermediate) host, which is in some instances a mammalian species (Watkins *et al.* 2006). *Hepatozoon* sp is usually transmitted by ingestion of the invertebrate host, but in the last years some studies have shown the experimental transmission of this protozoa in reptiles by mosquitoes (Adham *et al.* 2007; Sloboda *et al.* 2007) or in mammals by injection of the sporozoites recovered from ticks like *Amblyomma ovale* (Forlano *et al.*, 2005).

In a previous study performed by Giménez *et al* (2009) some zoonotic agents (Piroplasmida and *Hepatozoon* sp) were characterized in domestic and wild mammals from Northern Spain. In the present work such information is completed with a study on pathogens found in arthropods from wildlife in Burgos (Spain). Organisms such as *Rickettsia* spp and hemoprotezoa (piroplasmida and *Hepatozoon* spp) were identified by PCR and sequencing. These pathogens are important because they may cause disease either in animal and man. In

1
2
3
4 addition, detailed information concerning vectors and reservoirs is essential to implement
5
6 appropriate control measures (Torina et al, 2007).
7
8
9

10 11 **MATERIALS AND METHODS**

12
13 **Study area.** The study was carried out in the region of the Merindades (Burgos), that is
14 located in North West of Spain (42° 55' 52'' N, 3° 29' 2'' W). Summer temperature ranges
15 between 16-20°C and winter temperatures range between 2-5°C. The rainfall is usually high in
16 winter and ranges from 900-1.100 mm/year. It is a mainly rural area, but recreational activities
17 attracting non-residents have increased over the last few years.
18
19
20
21
22
23
24

25
26 **Animal samples.** Forty two wild animals (belonging to 13 species, see table 1 for details
27 on species and number of animals studied) were collected during the period between June 2006
28 and September 2007.
29
30
31
32

33 Wild animals were live-trapped, captured or in some instances found dead (in the latter
34 case death was due to road accidents). In all cases mammals were combed for ectoparasites
35 such as ticks, fleas, lice or mites. All these invertebrates were kept in 70% ethanol in sterile
36 tubes until further processing. Arthropods were identified on the basis of morphometric
37 characteristics. The keys used to identify fleas were those of Beacornu and Launay (1990); for
38 ticks, Estrada-Peña (2004) and for mites were used those of Baker (1999).
39
40
41
42
43
44
45
46

47 **DNA extraction, PCR, and Sequencing.** Samples were taken from 70% ethanol and
48 were rinsed in distilled water before being dried on sterile filter paper. DNA was extracted from
49 arthropods using alkaline hydrolysis, as described previously by Shouls *et al.* 1999 and Sousa *et*
50 *al.*, 2006). Whenever possible, DNA was extracted from pooled samples of 12 specimens of the
51 same arthropod species (all recovered from a single host). DNA of *Rickettsia* genera was
52 detected by amplification of citrate synthase (*gltA*) gene using the primers RpCs 1258/RpCs
53
54
55
56
57
58
59
60

1
2
3
4 877 that amplify a 381-bp fragment, 190-kDa protein (*ompA*) gene, using Rr 190.70p/Rr
5
6 190.602n primers pair which amplifying a 532-bp fragment (Regnery et al. 1991) and *ompB*
7
8 gene using 120-M59/120-807 primers that amplifying a 833-bp fragment (Roux and Raoult
9
10 2000).
11
12

13
14 Piroplasmids (*Babesia* sp and *Theileria* sp) were detected using the Universal *Babesia-*
15
16 *Theileria* primers BT1-F/BT1-R which amplifies a fragment of approximately 400-bp of the
17
18 18s rRNA gene (Criado-Fornelio et al, 2006). For the detection of *Hepatozoon*, primers
19
20 HEP1/HEP 4 were employed. These amplify a fragment of 660 bp of the 18s rRNA gene
21
22 (Criado-Fornelio *et al.* 2006). Negative and positive controls were included in all experiments.
23
24 Positive amplicons were purified with QIAquick Spin PCR purification kit (Qiagen, Hilden,
25
26 GmbH, Germany) and sequenced using an ABI 3130 automated sequencer (Applied
27
28 Biosystems, Foster City, CA, USA). The sequences were edited using the software Lasergene
29
30 (DNASTAR, inc., Madison, USA). The sequences were edited using the software Lasergene
31
32 (DNASTAR, inc., Madison, USA), the homology searches of amplicons were aligned with
33
34 corresponding sequences of other *Rickettsia*, *Babesia*, *Hepatozoon* species available in
35
36 GenBank/EMBL database, using the BLASTN software (Altschul *et al* 1990, Burland 2000).
37
38
39
40
41

42 RESULTS

43
44 A total of 258 arthropods were collected from 42 wild animals (details on invertebrate
45
46 species found are shown in Table 1). These included 107 ticks (16 adults [14.95%], 13 nymphs
47
48 [12.15%]), 78 larvae [73.89%]), 76 fleas and 73 mites (48 trombiculids, 25 not trombiculids).
49
50

51
52 The most prevalent flea species was *Paraceras melis* (32%), followed by *Ctenophthalmus*
53
54 sp. (22.6%), *Ctenocephalides felis* (17.3%) and *Ceratophyllus sciurorum* (17.3%). Other less
55
56 frequent flea species accounted for the remaining 10.8%. All of the trombiculids found
57
58 belonged to the genus *Neotrombicula*, (34.2%) and the other mites (non-trombicula) were
59
60

1
2
3
4 classified as *Laelaps agilis* (65.8%). In ticks, *Ixodes ricinus* was the most frequent species
5 (76.6%), followed by *Ixodes hexagonus* (22.4%) and *Ixodes trianguliceps* (0.93%).
6
7

8
9 Concerning microbiological and parasitological diagnostic, two fleas were infected by
10 identical rickettsia isolates. BLASTN sequence comparison showed that in both cases the two
11 studied genes (*gltA* - fragment of 381 bp and *ompB* - fragment of 825 bp) were 100% identical
12 to *R. felis* (AF540555). Both isolates were obtained in *Ctenocephalides felis* (1 individual and 1
13 pooled sample). These fleas had been recovered from two different *Martes foina* (beech
14 marten).
15
16
17
18
19
20
21
22

23 With regard to hemoprotozoa, *Hepatozoon* DNA was found both in fleas and mites. In
24 fleas, *Hepatozoon* sp (100% identity to *Hepatozoon* sp BV2 - AY600625) was diagnosed in two
25 specimens of *Ctenophthalmus* sp (recovered from bank vole - *Myodes glareolus*). A different
26 *Hepatozoon* sp isolate (100% identity to *Hepatozoon* sp red squirrel EF222259) was found in 1
27 DNA pool of *Ceratophyllus sciurorum* (recovered from red squirrel - *Sciurus vulgaris*). In
28 mites, a *Hepatozoon* isolate (100% identity to *Hepatozoon* sp BV2 AY600625) was found in a
29 DNA pool from *Neotrombicula* sp mites (recovered from bank vole - *Myodes glareolus*).
30
31 *Theileria annae* was found in ticks. Two isolates (100% identity to AY150069) were diagnosed
32 in *Ixodes ricinus* larvae (from wood mouse - *Apodemus sylvaticus*) and also in adult *Ixodes*
33 *hexagonus* (from red fox - *Vulpes vulpes*).
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 DISCUSSION

51 Changes in human habits or in the ecology of some reservoir hosts have contributed to a
52 closer contact of humans and arthropods vectors. This may have facilitated the spreading of
53 some emerging zoonoses. Defining vector species in a particular area is of the foremost
54 importance for disease control. In the present work, some putative vector species have been
55
56
57
58
59
60

1
2
3
4 found in a population of parasitic arthropods in Burgos. Fleas (from genera *Archeopsylla*,
5
6 *Ctenophthalmus* and *Ctenocephalides*) have been found to be likely rickettsia carriers for
7
8 domestic animals, as previously pointed out by other authors (either in Spain or elsewhere:
9
10 Marquez *et al*, 2002, Rolain *et al*, 2003; Bitam *et al*, 2006; Sousa *et al*, 2006). However, we
11
12 must underline that the present study is the first finding of *R. felis* in fleas (*Ctenocephalides*
13
14 *felis*) from wild animals in Spain. In our study the prevalence of Rickettsiae in *Ctenocephalides*
15
16 *felis* from wildlife animals represented at least a 15%, whilst prevalence in fleas of domestic
17
18 mammals ranged from 26.4 (Blanco *et al*, 2006) to 54.17% (Márquez *et al*, 2006). Positive fleas
19
20 were obtained from beech martens, this fact probably should be considered anecdotic but it is
21
22 interesting to mention that these wild mammals may approach human settlements in search of
23
24 food (Villoria *et al*, 2008), and it is possible the flea infection transferred from domestic
25
26 animals (cat or dog to beech marten). To our knowledge, this is the second report of molecular
27
28 detection of *R. felis* from fleas obtained from wild animals, other than wild rodents, in Europe.
29
30 In Portugal and Algeria, *R. felis* was found in the pulicid flea *Archeopsylla erinacei* from
31
32 hedgehogs (Sousa *et al*, 2006; Bitam *et al*, 2006). By this reason the possibility of transmission
33
34 to man by flea bite should not be disregarded. The interferences between sylvatic and domestic
35
36 cycles might influence prevalence infection in peridomestic animals, thus increasing the risk of
37
38 man exposure. Ticks such as *I. ricinus* and *I. hexagonus* have been found to be transmitters for
39
40 different species of rickettsiae (Schouls *et al*, 1999). In contrast, we failed to detect any
41
42 rickettsiae in the tick specimens analyzed. Other Acari like *Trombiculidae* may be responsible
43
44 of rickettsial transmission, but data on their vectorial ability are scarce in Spain. However, Choi
45
46 *et al* (2007) reported rickettsias belonging to spotted fever group (SFG) and typhus group (TG) in
47
48 these mites.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 Hemoprotozoa present in arthropods have been scarcely studied in Spain by molecular
5 methods. Thus, the present study is the first report of *Hepatozoon* sp in trombiculids or fleas of
6
7 wild mammals. Since no analysis of the vectorial capacity of these arthropods has been done in
8
9 the present study, the definitive hosts for *Hepatozoon* sp “BV2”/“red squirrel” remain
10
11 uncertain. Smith (1996) in his review of *Hepatozoon* species of mammals mentioned the
12
13 presence of *H. sylvatici* in bank voles and *Laelaps agilis* (mite). Molecular procedures showed
14
15 the existence of hepatozoons in Spanish bank voles by Criado-Fornelio *et al* (2006), and in
16
17 trombiculid mites (present work-prevalence 2%). Thus, the latter are likely definitive hosts. The
18
19 fact that fleas (in our study *Ctenophthalmus* sp with a prevalence of 11.7%) from bank voles
20
21 harbored the same parasite is not surprising, since these arthropods may easily feed on several
22
23 hosts (Service, 1996), thus increasing the chances of finding infected specimens. Concerning
24
25 *Hepatozoon* sp red squirrel, it has been found in a flea (*Ceratophyllus sciurorum* with a
26
27 prevalence of 7.6%), but this does not demonstrate vectorial capacity. Smith (1996) pointed out
28
29 that *H. griseisciuri* was found in squirrels and mites as well, so that previous findings do not
30
31 point out to fleas as the likely definitive hosts. Finally, it seems that *Hepatozoon* species from
32
33 arthropod species parasitizing Sciuridae or rodents have little chances to infect domestic
34
35 animals (particularly cats and dogs), and their only potential risk as pathogens remains only for
36
37 wildlife, in agreement with data published by Criado-Fornelio *et al* (2006).
38
39
40
41
42
43
44
45
46

47 Molecular methods revealed the existence of *Theileria annae* infections in *Ixodid* ticks.
48
49 This is in agreement with the hypothesis of Camacho *et al* (2003), who suggested that *Ixodidae*
50
51 (particularly *I. hexagonus*) was a good candidate vector for the protozoa (Camacho *et al*, 2003).
52
53 This is in agreement with findings in the present study, where one specimen of *I. hexagonus*
54
55 was infected by *Theileria annae* (prevalence 4.16%). The tick was recovered from fox, which
56
57 has been found to be frequently infected by piroplasmida in Spain (Criado-Fornelio *et al*, 2003,
58
59
60

1
2
3
4 and Giménez *et al*, 2009). Since foxes have been seen many times visiting human settlements,
5
6 they may carry infected ticks close to domestic canids. Although there had been no reports of
7
8 human infections caused by these protozoa, this possibility cannot be totally disregarded
9
10
11
12 (Camacho *et al*, 2001 and 2003).

13
14 Our results emphasize the potential risk of arthropods-transmitted infections in this study
15
16 area. Further studies are must be performed in the same area to determine the vectorial capacity
17
18 of arthropod species. These data are essential for the development of future control campaigns
19
20 in Spain or elsewhere.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

- 1
2
3
4
5
6
7 - Adham FK, Gabre RM, Ayaad TA, Galal FH. Experimental transmission of *Hepatozoon*
8
9 *gracilis* (Wenyon, 1909) com. nov., in its natural host the bean skink lizard (*Mabuza*
10 *quinquetaeniata quinquetaeniata*) and vector *Culex (C.) pipiens* (Diptera: *Culicidae*). J Egypt
11
12 Soc Parasitol. 2007; 37: 1199-212.
13
14
15
16 - Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. J Mol Biol 1990; 215: 403-410.
17
18 - Baker. A.S. (1999). *Mites and ticks of domestic animals*. The Natural History Museum. 240 pp.
19
20 - Beaucournu, JC. y H.Launay (1990). *Les puces de France et du bassin mediterraneen*
21
22 *occidental*. Faune de France 76. París. 548 pp.
23
24
25
26 - Bitam I, Parola P, De La Cruz KD, Matsumoto K, Baziz B, Rolain JM, Belkaid M, Raoult D.
27
28 First molecular detection of *Rickettsia felis* in fleas from Algeria. Am J Trop Med Hyg. 2006;
29
30 74: 532-535.
31
32
33 -Blanco JR, Oteo JA. Rickettsiosis in Europe. Ann N Y Acad Sci 2006; 1078: 26-36.
34
35 -Blanco JR, Pérez-Martínez L, Vallejo M, Santibañez S, Portillo A, Oteo JA. Prevalence of
36
37 *Rickettsia felis*-like and Bartonella spp in *Ctenocephalides felis* and *Ctenocephalides canis* from
38
39 La Rioja (Northern Spain). Ann N Y Acad Sci 2006; 1078: 270-274.
40
41
42 - Blaschitz M, Narodoslavsky-Gföller M, Kanzler M, Stanek G, Walochnik J. Babesia species
43
44 occurring in Austrian *Ixodes ricinus* ticks. Appl Environ Microbiol. 2008; 74: 4841-4846.
45
46
47 -Burland TG. DNASTAR's Lasergene sequence analysis software. Methods Mol Bio 2000;
48
49 132: 71-91.
50
51
52 - Camacho AT, Pallas E, Gestal JJ, Guitián FJ, Olmeda AS, Goethert HK, Telford SR. Infection
53
54 of dogs in north-west Spain with a *Babesia microti*-like agent. Vet Rec. 2001; 149: 552-555.
55
56
57
58
59
60

- 1
2
3
4 - Camacho AT, Pallas E, Gestal JJ, Guitián FJ, Olmeda AS, Telford SR, Spielman A. *Ixodes*
5
6 *hexagonus* is the main candidate as vector of *Theileria annae* in northwest Spain. Vet Parasitol.
7
8 2003; 112: 157-163
9
- 10
11 - Choi YJ, Lee EM, Park JM, Lee KM, Han SH, Kim JK, Lee SH, Song HJ, Choi MS, Kim IS,
12
13 Park KH, Jang WJ. Molecular detection of various rickettsiae in mites (acari: *trombiculidae*) in
14
15 southern Jeolla Province, Korea. Microbiol Immunol 2007; 51: 307-312.
16
17
- 18
19 - Criado-Fornelio A, Ruas JL, Casado N, Farias NA, Soares MP, Müller G, Brumt JG, Berne
20
21 ME, Buling-Saraña A, Barba-Carretero JC. New molecular data on mammalian Hepatozoon
22
23 species (Apicomplexa: Adeleorina) from Brazil and Spain. J Parasitol. 2006; 92:93-9.
24
25
- 26 - Estrada – Peña, A., Camicas, J.L. y A.R. Walker (2004). Ticks of domestic animals in the
27
28 mediterranean region: A guide to identification of species. Universidad de Zaragoza. 131pp.
29
30
- 31 - Forlano M, Scofield A, Elisei C, Fernandes KR, Ewing SA, Massard CL. Diagnosis of
32
33 *Hepatozoon spp.* in *Amblyomma ovale* and its experimental transmission in domestic dogs in
34
35 Brazil. Vet Parasitol. 2005; 134: 1-7.
36
37
- 38 - Giménez C, Casado N, Criado-Fornelio A, Álvarez de Miguel F, Domínguez-Peñañiel G. A
39
40 molecular survey of Piroplasmida and *Hepatozoon* isolates from domestic and wild animals in
41
42 Burgos (northern Spain). Vet Parasitol. 2009; 162: 147-150.
43
44
- 45 - Márquez FJ, Muniain MA, Pérez JM, Pachón J. Presence of *Rickettsia felis* in the cat flea
46
47 from southwestern Europe. Emerg Infect Dis. 2002; 8:89-91.
48
49
- 50 - Márquez FJ, Muniain MA, Rodríguez-Liebana JJ, Toro MD, Bernabeu-Wittel M, Pachon AJ.
51
52 Incidence and distribution pattern of *Rickettsia felis* in peridomestic fleas from Andalusia,
53
54 Southeast Spain. Ann N Y Acad Sci 2006; 1078: 344-346.
55
56
- 57 - Regnery RL, Spruill CL, Plikaytis BD. Genotypic Identification of *Rickettsiae* and estimation
58
59 of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 1991; 173:
60

1
2
3
4 1576-1589.

5
6
7 - Rolain JM, Franc M, Davoust B, Raoult D. Molecular detection of *Bartonella quintana*, *B.*
8
9 *koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas,
10
11 France. Emerg Infect Dis. 2003; 9: 338-342.

12
13
14 - Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene
15
16 encoding the outer-membrane protein rOmpB (*ompB*). Int J Syst Evol Microbiol 2000; 50:
17
18 1449-1455.

19
20
21 - Schouls LM, Van de Pol I, Rijpkema SGT, Schot CS. Detection and identification of
22
23 *Ehrlichia*, *Borrelia burgdorferi* Sensu Lato, and *Bartonella* species in Dutch *Ixodes ricinus*
24
25 ticks. J Clin Microbiol 1999; 37: 2215-2222.

26
27
28 -Service, M.W. Fleas (Siphonaptera). In Medical Entomology, Ed. Chapman and Hall,,London,
29
30 first edition.. 1996: pp. 175-188.

31
32
33 - Sloboda M, Kamler M, Bulantová J, Votýpka J, Modrý D. A new species of *Hepatozoon*
34
35 (Apicomplexa: *Adeleorina*) from *Python regius* (Serpentes: *Pythonidae*) and its experimental
36
37 transmission by a mosquito vector. J Parasitol. 2007; 93:1189-1198.

38
39
40 - Sloboda M, Kamler M, Bulantová J, Votýpka J, Modrý D. Rodents as intermediate hosts of
41
42 *Hepatozoon ayorgbor* (Apicomplexa: *Adeleina*: *Hepatozoidae*) from the African ball python,
43
44 *Python regius*. Folia Parasitol (Praha). 2008; 55: 13-16.

45
46
47 - Smith, T.G. The genus *Hepatozoon* (Apicomplexa: Adeleina). J. Parasitol. 1996: 82: 565-585.

48
49
50 -Sousa R, Edouard-Fournier P, Santos –Silva M, Amaro F, Bacellar F, Raoult D. Molecular
51
52 detection of *Rickettsia felis*, *Rickettsia typhi*, and two genotypes closely related to *Bartonella*
53
54 *elizabethae*. Am J Trop Med Hyg 2006; 75: 727-731.

1
2
3
4 Torina, A., Vicente, J., Alongi, A., Scimeca, S., Turlá, R., Nicosia, S., Di Marco, V.,
5
6 Caracappa, S., de la Fuente, J. Observed prevalence of tick-borne pathogens in domestic
7
8 animals in Sicily, Italy during 2003-2005. *Zoon. Pub. Health*, 2007; 54: 8-15.
9

10
11 - Villoria, J.S., Sánchez, M., Fombellida, I, Herrero, A., Sánchez, A. Lista preliminar de los
12
13 vertebrados continentales de Cantabria. *Locustella* 2008: 1: 7-24
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Table 1- Ectoparasites identified in wild mammals.

Mammal species (animals studied)	Tick species (no., stage*)	Mite species (no.)	Flea species (no.)	Louse species (no.)
<i>Arvicola terrestris</i> (2)	None	None	<i>Ctenophtalmus sp</i> (1 pool)	None
<i>Apodemus flavicollis</i> (3)	<i>Ixodes ricinus</i> (1 L)	<i>Laelaps agilis</i> (1+ 1 pool)	<i>Ctenophtalmus sp</i> (2)	None
<i>A.sylvaticus</i> (11)	<i>Ixodes trianguliceps</i> (1 A) <i>Ixodes ricinus</i> (16 L+4 pool L)	<i>Laelaps agilis</i> (1 pool) <i>Neotrombicula sp</i> (12)	<i>Ctenophtalmus sp.</i> (1)	None
<i>Capreolus capreolus</i> (5)	<i>Ixodes ricinus</i> (1 A+1 pool A)	None	None	None
<i>Myodes glareolus</i> (4)	None	<i>Neotrombicula sp</i> (12+2 pool)	<i>Ctenophtalmus sp.</i> (2)	None
<i>Martes foina</i> (2)	<i>Ixodes hexagonus</i> (5 N)	None	<i>Ctenocephalides felis</i> (1+ 1 pool) <i>Pulex irritans</i> (1) <i>Paraceras melis</i> (1 pool)	<i>Trichodectes melis</i> (1)

<i>Martes martes</i> (2)	<i>Ixodes hexagonus</i> (2 N)	None	<i>Ceratophyllus sciurorum</i> (1)	None
<i>Meles meles</i> (1)	None	None	<i>Paraceras melis</i> (1 pool)	<i>Trichodectes melis</i> (1)
<i>Putorius putorius</i> (1)	<i>I. hexagonus</i> (1 pool N)	None	None	None
<i>Sciurus vulgaris</i> (2)	<i>Ixodes ricinus</i> (1 N)	None	<i>Ceratophyllus sciurorum</i> (1 pool)	None
<i>Strix aluco</i> (1)	<i>Ixodes ricinus</i> (2 N)	None	None	None
<i>Talpa occidentalis</i> (3)	None	None	<i>Palaeopsylla minor</i> (3)	None
<i>Vulpes vulpes</i> (5)	<i>Ixodes hexagonus</i> (2 N; 3 A) <i>I. ricinus</i> (1 N)	None	<i>Pulex irritans</i> (3) <i>Ctenocephalides canis</i> (2)	None

- = Abbreviations: L= larvae; N=nymph; A=Adult
- Pool= 12 specimens of the same arthropod species