



Short communication

Infestation of *Rhipicephalus sanguineus* sensu lato on cats in MaltaSándor Hornok^{a,*}, Andrea Grima^a, Nóra Takács^a, Jenő Kontschán^b^a Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary^b Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

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ABSTRACT

Studies on cats as hosts of *Rhipicephalus sanguineus* sensu lato (s.l.) are scarce. Cats are regarded as infrequent hosts of this species complex, and usually only when dogs are also present. In order to compare the occurrence of developmental stages and mitochondrial DNA haplotypes of *R. sanguineus* s.l. on cats and other domestic or synanthropic mammalian species, 540 ticks were collected from cats, dogs, hedgehogs and one goat. Collections were made from April to September in 2016 and 2017, from 20 locations in Malta in southern Europe. The sampling sites included six cat colonies, where no dogs were present. Compared to adults, significantly more immatures of *R. sanguineus* s.l. were found on cats (123 larvae and nymphs versus 10 adults) than on dogs (190 larvae and nymphs versus 173 adults). Furthermore, compared to nymphs, significantly more larvae of *R. sanguineus* s.l. were found on cats (50 larvae versus 73 nymphs) than on dogs (11 larvae versus 179 nymphs). Adult ticks predominated on male dogs (42 adults versus 28 larvae or nymphs), whereas immatures were significantly more abundant compared to adult ticks on female dogs (142 larvae or nymphs versus 80 adults). Similarly, immature as compared with adult ticks were significantly more likely to occur on female cats (72 immature ticks versus 1 adult) in comparison with male cats (46 immature ticks versus 8 adults). Moreover, *R. sanguineus* s.l. larvae were found significantly more frequently as compared with nymphs on female cats (38 larvae versus 34 nymphs) than on male cats (12 larvae versus 34 nymphs). To confirm morphological identification and to compare mitochondrial markers (cytochrome *c* oxidase subunit 1 [*cox1*] and 16S rRNA genes) of ticks across hosts, 57 ticks were analysed. The amplified parts of the *cox1* and 16S rRNA genes of *R. sanguineus* s.l. collected from various hosts showed 100% sequence identity with each other and with those in GenBank from the middle to western Mediterranean Basin. In conclusion, the present study highlights that cats can be important hosts of the immature life stages of *R. sanguineus* s.l., even in the absence of dogs. This finding has veterinary-medical significance, because stray cats and free-roaming cats may transport immature stages of *R. sanguineus* s.l. into gardens, i.e. near dogs and humans.

1. Introduction

Hard ticks (Acari: Ixodidae) are regarded as the most important ectoparasites of terrestrial vertebrates in the temperate zone (Jongejan and Uilenberg, 2004), owing to the broad geographical distribution of several species with high veterinary-medical importance. Among them, *Rhipicephalus sanguineus* sensu lato (s.l.) is probably the most widespread group of tick species in the world (Dantas-Torres, 2010). Although it is adapted to complete its life cycle involving only dogs (its principal host species), it may also infest small mammals and livestock animals (Filippova, 1997; Estrada-Peña et al., 2004; Dantas-Torres, 2010). Interestingly, although cats are frequently listed as the hosts of *R. sanguineus* s.l. second to dogs, actual accounts of *R. sanguineus* s.l. infestation on cats are scarce. Studies conducted in the New World attest to a usually low prevalence of infestation (Akucewich et al., 2002;

Mendes-de-Almeida et al., 2011; Thomas et al., 2016). In addition, the general view is that domestic cats are infrequent hosts of this species complex (Hoogstraal, 1956) and that cats usually harbor *R. sanguineus* s.l. only when dogs are also present in their environment (Estrada-Peña et al., 2004; Uspensky, 2009; Dantas-Torres, 2010).

In several geographical regions, the host spectrum of *R. sanguineus* s.l. is not well known. In Europe, *R. sanguineus* s.l. is endemic to southern countries (Lorusso et al., 2010; Pennisi et al., 2015; Hornok et al., 2017), although there are reports on its establishment north of the Mediterranean Basin (Hansford et al., 2015; Hornok et al., 2017). The three islands of the Republic of Malta are situated south of mainland Europe and north of Africa, thus representing climatic conditions that favour *R. sanguineus* s.l. Accordingly, this group of tick species was recently recorded from dogs in Malta (Hornok et al., 2017; Licari et al., 2017).

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The aim of this work was to broaden the knowledge of the host spectrum of the *R. sanguineus* s.l. group. Therefore, ticks were collected from four mammalian species in Malta. Host selection focused primarily on dogs and cats. Cats are known to bring ticks into the human environment (Sadek, 2001), but despite this, data on the occurrence of *R. sanguineus* s.l. on cats in Europe and the Mediterranean Basin are limited. In particular, during previous surveys of cat ectoparasites, only few cats have been found infested with *R. sanguineus* s.l., either with only adult ticks (Salant et al., 2014; Pennisi et al., 2015) or with the developmental stage not reported (Ortuño et al., 2008; Lefkaditis et al., 2015).

We use the nomenclature *R. sanguineus* s.l. because the taxonomy of this species complex is unresolved. Hoogstraal (1956) discussed morphological variations and biological strains in this species complex, and later its biosystematics was evaluated with cross-breeding experiments (Pegram et al., 1987). More recently, *R. sanguineus* s.l. has been found to consist of well-separated genetic lineages (Dantas-Torres et al., 2013). Therefore, in this study, we use *R. sanguineus* s.l. although only one haplotype has been reported in Malta (Hornok et al., 2017).

2. Materials and methods

2.1. Tick collection and morphological identification

The sampling period was chosen to encompass summer months, when immature stages and adults are active (Lorusso et al., 2010). Ticks were removed from 41 dogs, 31 cats, two hedgehogs, and one goat from April to September in 2016 and 2017, in 20 locations in Malta (data not shown). In addition, ticks were collected with tweezers from the ground in places where dogs and cats live. Sampled dogs were kept at houses, whereas cats both at houses and in six cat colonies, where no dogs were present. These cat colonies consist of stray cats, which are spayed/castrated and microchipped during their prolonged stay. All ticks were removed during regular veterinary care, therefore no ethical permission was needed, but verbal consent was obtained from the owners.

Specimens were stored in 96% ethanol, and their taxon (*R. sanguineus* s.l.) was identified by morphological characters using a recent description (Estrada-Peña et al., 2017). In particular, adults of *R. sanguineus* s.l. were identified to genus based on their hexagonal basis capituli. Both sexes had narrow dorsal prolongation of their spiracular plates (longer in males); the adanal plates of males were not pointed medially, and the scutum of females was as wide as long. Identification of nymphs was based on scutal dimensions, scutal-alloscutal setal length and medially serrate posterior palpal hairs; and that of larvae was based on scutal-alloscutal hairs (Filippova, 1997) as shown in Fig. 1. Pictures were taken with a VHX-5000 digital microscope (Keyence Co., Osaka, Japan).

2.2. Molecular analyses

DNA was extracted from 57 representative individual ticks (more than 10% of all ticks collected) with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer and Proteinase-K at 56 °C.

The cytochrome c oxidase subunit I (*cox1*) gene was chosen as the primary target for molecular identification and comparison of 11 tick specimens, on account of the suitability of this target as a DNA-barcode sequence for tick species identification (Lv et al., 2014). These 11 ticks were selected from hosts not yet reported to harbor *R. sanguineus* s.l. in Malta (i.e., four ticks from cats, six from hedgehogs and one from goat). The PCR was modified from Folmer et al. (1994) and amplifies an approximately 710 bp long fragment of the gene. The primers HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') were used in a reaction volume of 25 µl, containing 1 U (0.2 µl) HotStarTaq Plus DNA

polymerase, 2.5 µl 10 × CoralLoad Reaction buffer (including 15 mM MgCl₂), 0.5 µl PCR nucleotide Mix (0.2 mM each), 0.5 µl (1 µM final concentration) of each primer, 15.8 µl ddH₂O and 5 µl template DNA. For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 48 °C for 1 min and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min. To complement the results obtained with the *cox1* gene (of 11 ticks), and to confirm the morphological identification for another 46 ticks (larvae and nymphs: 15 from dogs, 31 from cats), another PCR was used to amplify an approximately 460 bp fragment of the 16S rRNA gene of Ixodidae (Black and Piesman, 1994), with the primers 16S + 1 (5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3') and 16S-1 (5'-CCG GTC TGA ACT CAG ATC AAG T-3'). Other reaction components, as well as cycling conditions were the same as above, except for annealing at 51 °C.

PCR products were visualized with ethidium-bromide on a 1.5% agarose gel. Purification and sequencing was done by Biomi Inc. (Gödöllő, Hungary). Sequences were manually edited and compared with the BLASTn program (<https://blast.ncbi.nlm.nih.gov>). Representative sequences were submitted to GenBank; accession numbers for ticks from cat, goat, and hedgehog are MG855656-MG855658 (*cox1* gene) and MG855660-MG855662 (16S rRNA gene), respectively. The ratios of haplotypes were compared by Fisher's exact test (condition of significance: $P < 0.05$).

3. Results

Altogether 540 ticks of the *R. sanguineus* group were collected: 363 (67.2%) from dogs, 133 (24.6%) from cats, eight (1.5%) from hedgehogs, one from a goat and 35 from the environment. No other tick species were found on these animals.

3.1. Host-associations of *R. sanguineus* s.l.

All stages and sexes of *R. sanguineus* s.l. (larvae, nymphs, males and females) occurred on both dogs and cats (Table 1). There was no significant association between the host species (dog versus cat) or the sex of these hosts (males versus females) and the presence of either male or female ticks. However, compared to adults, significantly more immatures of *R. sanguineus* s.l. were found on cats (123 larvae and nymphs versus 10 adults) than on dogs (190 larvae and nymphs versus 173 adults) ($P < 0.00001$). Furthermore, compared to nymphs, significantly more larvae of *R. sanguineus* s.l. were found on cats (50 larvae versus 73 nymphs) than on dogs (11 larvae versus 179 nymphs) ($P < 0.00001$). These differences remained highly significant even when only dogs and cats kept in similar environments (i.e., at houses) were taken into account (Table 1, household cats: 58 larvae and nymphs versus 10 adults, 27 larvae versus 31 nymphs).

Concerning the distribution of *R. sanguineus* s.l. life stages between male and female hosts (Table 1), adult ticks predominated on male dogs (42 adults versus 28 larvae or nymphs), whereas immatures were more abundant compared to adult ticks on female dogs (142 larvae or nymphs versus 80 adults). This was a statistically significant association ($P = 0.0005$). Similarly, immature as compared with adult ticks were significantly more likely to occur on female cats (72 immature ticks versus 1 adult) in comparison with male cats (46 immature ticks versus 8 adults) ($P = 0.005$). Moreover, *R. sanguineus* s.l. larvae were found more frequently as compared with nymphs on female cats (38 larvae versus 34 nymphs) than on male cats (12 larvae versus 34 nymphs) ($P = 0.005$).

When examining data in the latter context separately according to where animals were housed, significantly more immatures of *R. sanguineus* s.l. were found on male cats in colonies (31 on male cats versus 34 on female cats) than on male cats at houses (15 on male cats versus 38 on female cats). Adults of *R. sanguineus* s.l. were not found on cats in colonies, as contrasted to household cats.

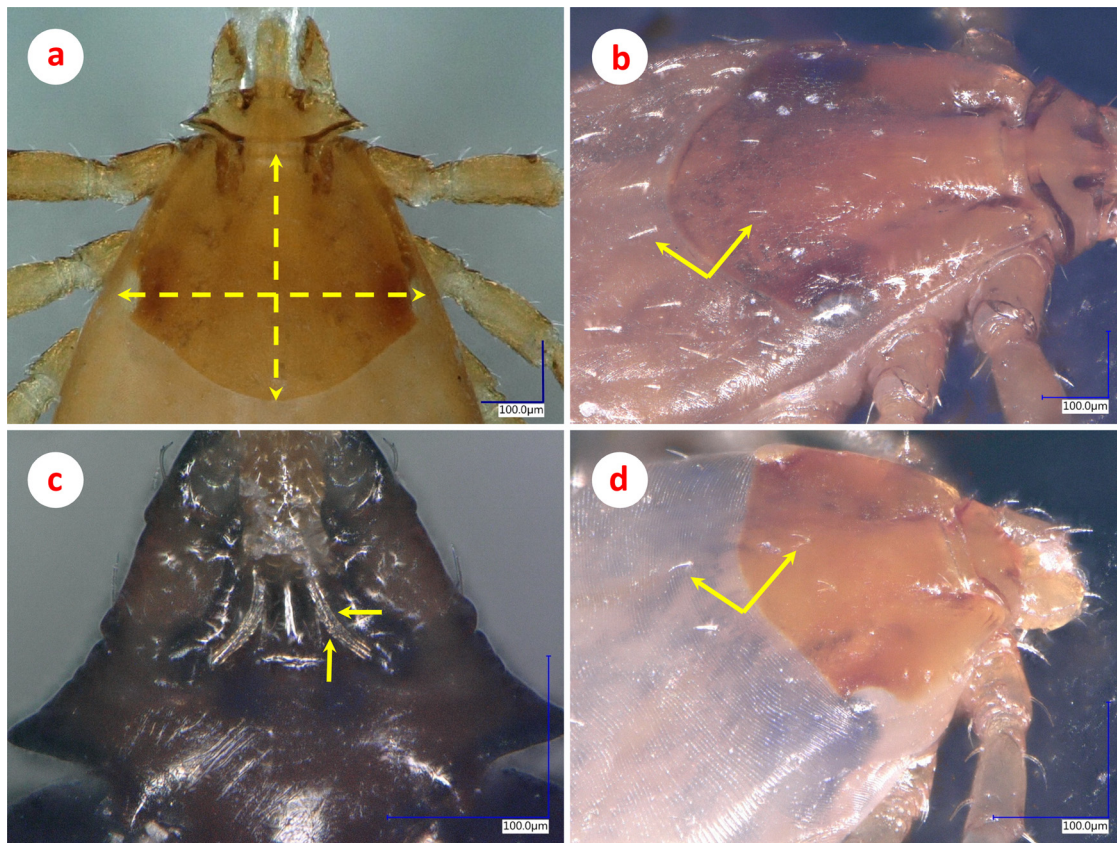


Fig. 1. Diagnostic features used for identification of *Rhipicephalus sanguineus* immature stages and to distinguish them from those of *R. turanicus*, according to [Filippova \(1997\)](#). (a) Nymph: the scutum is broader than long; (b) Nymph: alloscutal hairs approximately twice as long as scutal hairs (arrows); (c) Nymph: posteromedial palpal seta medially serrated (vertical arrow), laterally smooth (horizontal arrow); (d) Larva: alloscutal hairs approximately one and a half times as long as scutal hairs (arrows).

In addition, one female *R. sanguineus* s.l. was removed from a goat, and two females, two males, four nymphs were collected from two hedgehogs.

3.2. Molecular identification and comparison of *R. sanguineus* s.l

Four specimens of *R. sanguineus* s.l. collected from cats had identical (630/630 bp = 100% identity) *cox1* gene sequences with each other, and with those collected from dogs in Malta during a previous study (GenBank: KX757902). Similarly, six specimens of *R. sanguineus* collected from hedgehogs and one removed from a goat had identical *cox1* gene sequences with the above ticks from cats. This was confirmed by the analyses of 16S rRNA gene sequences of the same samples, which also showed 100% (i.e., 405/405 bp) identity with each other and with a formerly reported *R. sanguineus* sequence (GenBank: KX793735) from

Malta. These ticks from Malta had 100% *cox1* or 16S rRNA gene sequence identity with *R. sanguineus* s.l. samples from the middle and western Mediterranean Basin, including Italy (GenBank: KX757904), Croatia (GenBank: KX757896) and Algeria (GenBank: KX757910).

4. Discussion

The main host for all stages of *R. sanguineus* s.l. is the dog, and hosts other than dogs are usually considered to be infested only when dogs are present ([Estrada-Peña et al., 2004](#); [Uspensky, 2009](#); [Dantas-Torres, 2010](#)). Thus, cats are regarded as occasional hosts of this tick species ([Akucewich et al., 2002](#); [Mendes-de-Almeida et al., 2007](#)). *Rhipicephalus sanguineus* s.l. larvae and nymphs may also attach to small mammals, such as rodents and rabbits ([Dryden and Payne, 2004](#); [Nicholson et al., 2009](#)) or humans ([Estrada-Peña and Jongejan, 1999](#)). The present study

Table 1
Distribution of *Rhipicephalus sanguineus* s.l. stages on dogs and cats.

Host species (n)	Sex of host (n)	Tick stage or sex							
		Larva		Nymph		Male		Female	
		in colony	at houses	in colony	at houses	in colony	at houses	in colony	at houses
Dog (n = 41)	Male (n = 9, at houses)	–	2	–	26	–	20	–	22
	Female (n = 20, at houses)	–	9	–	133	–	38	–	42
	unrecorded (n = 12, at houses)	–	–	–	20	–	24	–	27
Cat (n = 31)	Male (n = 8 in colony, 11 at houses)	6	6	25	9	–	5	–	3
	Female (n = 3 in colony, 7 at houses)	17	21	17	17	–	1	–	–
	unrecorded (n = 2, at houses)	–	–	–	5	–	–	–	1

offered the opportunity to compare the infestation prevalence of life stages of *R. sanguineus* s.l. between dogs and cats, for which literature data are scarce. Here, significantly more immature stages (especially larvae) of *R. sanguineus* s.l. were found on cats than on dogs. This is in contrast to some other reports, where only adults of this tick species were found on cats (Pennisi et al., 2015; Thomas et al., 2016). Nevertheless, it is also possible, that in some previous studies immature stages of *R. sanguineus* s.l. were overlooked, because they are very small and therefore easily missed on physical examination (Sadek, 2001).

One plausible explanation for the more likely occurrence of immature *R. sanguineus* s.l. stages on cats compared to dogs is that larvae and nymphs of *R. sanguineus* s.l. tend to occur close to hidden places associated with their moulting (Dantas-Torres, 2010), and cats are known to enter narrow (crawl-) spaces of houses (Loyd et al., 2013), unlike dogs. In addition, cats may more likely encounter *R. sanguineus* s.l. larvae on rodent prey animals compared to dogs, and some of these larvae (theoretically) may re-attach to a new host (cat) in the same stage (Shih and Spielman, 1993) or in the next stage. An alternative explanation is that cats have meticulous grooming habit (Sadek, 2001), and this allows them to remove adult ticks (which are larger) more effectively than small larvae or nymphs.

In either case, this tendency of infestation predominantly with larvae and nymphs of *R. sanguineus* s.l. confers high significance to the transportation of these stages by cats, because immature *R. sanguineus* s.l. are more likely to attach to (and to remain unnoticed on) human beings (Estrada-Peña and Jongejan, 1999; Uspensky, 2009). In addition, cats are known to harbor *R. sanguineus*-transmitted zoonotic pathogens (e.g., *Rickettsia conorii* and *R. massiliae*; Segura et al., 2014) and to bring ticks into the human environment (Sadek, 2001). Thus, the present findings should be taken into account when assessing zoonotic diseases associated with free-roaming cats (Gerhold and Jessup, 2013).

Concerning the distribution of *R. sanguineus* s.l. stages between male and female hosts, adult ticks predominated on male dogs, whereas immature stages were more abundant on female dogs, which was a statistically significant association. Similarly, immature ticks were significantly more likely to occur on female cats in comparison with male cats. Moreover, findings of *R. sanguineus* s.l. larvae reflected a tendency that this stage attaches more frequently to female cats than to male cats. *Rhipicephalus sanguineus* s.l. is an endophilic tick species (Dantas-Torres, 2010); therefore, immature stages tend to attach to animals using such sheltered environments more frequently or for a prolonged time, i.e., female dogs or cats as shown here (e.g., during gestation and lactation). On the contrary, male cats may roam more, for example in search of a female (Loyd et al., 2013).

Significantly more immature ticks were found on male cats in colonies than at houses, and adults of *R. sanguineus* s.l. were not found on cats in colonies, supporting the idea that restricted movements may promote infestation of cats with early developmental stages of *R. sanguineus* s.l. In contrast, adult ticks may predominate on free-roaming cats (Thomas et al., 2016). Taken together, the presence of *R. sanguineus* s.l. in all six evaluated cat colonies attest that this group of ticks will use cats as hosts even in the absence of dogs. This adds to other, already known aspects of the epidemiological importance of cat colonies (Roebeling et al., 2014).

Our finding of *R. sanguineus* s.l. on goats is consistent with the reported occurrence of this tick species on ruminants (Estrada-Peña et al., 2004; Zakkyeh et al., 2012). Hedgehogs are mainly parasitized by adults of this tick species in the Mediterranean region (Marié et al., 2012), in part also shown here.

The 100% identity of *cox1* (and 16S rRNA) gene sequences of *R. sanguineus* s.l. between specimens collected from different host species (cats, hedgehogs, goat) in the present study reflects the optional usage of a relatively broad host range and genetic exchange between relevant tick populations. In addition, samples analysed here were identical with those reported in some other parts of the middle-western part of the Mediterranean Basin (Hornok et al., 2017). Taking into account that *R.*

sanguineus s.l. is seldom reported from birds (Szabó et al., 2012), these data from islands (as exemplified by Malta) imply that continuous dispersal and consequent gene flow between *R. sanguineus* s.l. populations over larger geographical distances is not *per se* host-mediated, but rather human-mediated (as a result of pet travel, livestock trade).

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References

- Akuciewicz, L.H., Philman, K., Clark, A., Gillespie, J., Kunkle, G., Nicklin, C.F., Greiner, E.C., 2002. Prevalence of ectoparasites in a population of feral cats from north central Florida during the summer. *Vet. Parasitol.* 109, 129–139.
- Black, W.C., Piesman, J., 1994. Phylogeny of hard and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proc. Nat. Acad. Sci. U. S. A.* 91, 10034–10038.
- Dantas-Torres, F., Latrofa, M.S., Annoscia, G., Giannelli, A., Parisi, A., Otranto, D., 2013. Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. *Parasit. Vectors* 6, 213.
- Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasit. Vectors* 3, 26.
- Dryden, M.W., Payne, P.A., 2004. Biology and control of ticks infesting dogs and cats in North America. *Vet. Ther.* 5, 139–154.
- Estrada-Peña, A., Jongejan, F., 1999. Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Exp. Appl. Acarol.* 23, 685–715.
- Estrada-Peña, A., Bouattour, A., Camicas, J.-L., Walker, A.R., 2004. Ticks of Domestic Animals in the Mediterranean Region: A Guide to Identification of Species. Zaragoza University of Zaragoza Publishing House, pp. 1–137.
- Estrada-Peña, A., Pfäffle, M., Baneth, G., Kleinerman, G., Petney, T.N., 2017. Ixodoidea of the Western Palaearctic: a review of available literature for identification of species. *Ticks Tick-Borne Dis.* 8, 512–525.
- Filippova, N.A., 1997. Ixodid ticks of the subfamily Amblyomninae. *Fauna of Russia and Neighbouring Countries*, vol. 4. pp. 1–436 (in Russian).
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Gerhold, R.W., Jessup, D.A., 2013. Zoonotic diseases associated with free-roaming cats. *Zoonoses Public Health* 60, 189–195.
- Hansford, K.M., Pietzsch, M.E., Cull, B., Medlock, J.M., Wall, R., 2015. Overwintering of the brown dog tick in residential properties in England—raising awareness. *Vet. Rec.* 177, 156.
- Hoogstraal, H., 1956. African Ixodoidea. 1. Ticks of the Sudan with Special Reference to Equatoria Province and with Preliminary Review of Genera *Rhipicephalus*, *Margaropus* and *Hyalomma*. US Navy, Washington D.C, pp. 686–724.
- Hornok, S., Sándor, A.D., Tomanović, S., Beck, R., D'Amico, G., Kontschán, J., Takács, N., Görfö, T., Bendjedou, M.L., Földvári, G., Farkas, R., 2017. East and west separation of *Rhipicephalus sanguineus* mitochondrial lineages in the Mediterranean Basin. *Parasit. Vectors* 10, 39.
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129 (Suppl), S3–S14.
- Lefkaditis, M.A., Sossidou, A.V., Panorias, A.H., Koukeri, S.E., Paştıu, A.I., Athanasiou, L.V., 2015. Urban stray cats infested by ectoparasites with zoonotic potential in Greece. *Parasitol. Res.* 114, 3931–3934.
- Licari, E., Takács, N., Solymosi, N., Farkas, R., 2017. First detection of tick-borne pathogens of dogs from Malta. *Ticks Tick-Borne Dis.* 8, 396–399.
- Lorusso, V., Dantas-Torres, F., Lia, R.P., Tarallo, V.D., Mencke, N., Capelli, G., Otranto, D., 2010. Seasonal dynamics of the brown dog tick, *Rhipicephalus sanguineus*, on a confined dog population in Italy. *Med. Vet. Entomol.* 24, 309–315.
- Loyd, K.A., Hernandez, S.M., Abernathy, K.J., Shock, B.C., Marshall, G.J., 2013. Risk behaviours exhibited by free-roaming cats in a suburban US town. *Vet. Rec.* 173, 295.
- Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., Lin, X., 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasit. Vectors* 7, 93.
- Marié, J.L., Davoust, B., Socolovschi, C., Raoult, D., Parola, P., 2012. Molecular detection of rickettsial agents in ticks and fleas collected from a European hedgehog (*Erinaceus europaeus*) in Marseilles, France. *Comp. Immunol. Microbiol. Infect. Dis.* 35, 77–79.
- Mendes-de-Almeida, F., Labarthe, N., Guerrero, J., Faria, M.C., Branco, A.S., Pereira, C.D., Barreira, J.D., Pereira, M.J., 2007. Follow-up of the health conditions of an urban colony of free-roaming cats (*Felis catus* Linnaeus, 1758) in the city of Rio de Janeiro, Brazil. *Vet. Parasitol.* 147, 9–15.
- Mendes-de-Almeida, F., Crissiuma, A.L., Gershony, L.C., Willi, L.M., Paiva, J.P., Guerrero, J., Labarthe, N., 2011. Characterization of ectoparasites in an urban cat (*Felis catus* Linnaeus, 1758) population of Rio de Janeiro, Brazil. *Parasitol. Res.* 108, 1431–1435.
- Nicholson, W.L., Sonenshine, D.E., Lane, R.S., Uilenberg, G., 2009. Ticks (Ixodida). In: Mullen, G.R., Durden, L.A. (Eds.), *Medical and Veterinary Entomology*. Academic Press Elsevier Science, Amsterdam, pp. 493–542.
- Ortuño, A., Castellà, J., Criado-Fornelio, A., Bulng, A., Barba-Carretero, J.C., 2008. Molecular detection of a *Hepatozoon* species in stray cats from a feline colony in

- North-eastern Spain. *Vet. J.* 177, 134–135.
- Pegram, R.G., Keirans, J.E., Clifford, C.M., Walker, J.B., 1987. Clarification of the *Rhipicephalus sanguineus* group (Acari, Ixodoidea, Ixodidae). II. *R. sanguineus* (Latreille, 1806) and related species. *Syst. Parasitol.* 10, 27–44.
- Pennisi, M.G., Persichetti, M.F., Serrano, L., Altet, L., Reale, S., Gulotta, L., Solano-Gallego, L., 2015. Ticks and associated pathogens collected from cats in Sicily and Calabria (Italy). *Parasits Vectors* 8, 512.
- Roebling, A.D., Johnson, D., Blanton, J.D., Levin, M., Slate, D., Fenwick, G., Rupprecht, C.E., 2014. Rabies prevention and management of cats in the context of trap-neuter-vaccinate-release programmes. *Zoonoses Public Health* 61, 290–296.
- Sadek, T.P., 2001. Vector-associated zoonoses in cats. In: Lappin, L.R. (Ed.), *Feline Internal Medicine Secrets*. Hanley and Belfus Inc, Philadelphia, pp. 444–448.
- Salant, H., Mumcuoglu, K.Y., Baneth, G., 2014. Ectoparasites in urban stray cats in Jerusalem, Israel: differences in infestation patterns of fleas, ticks and permanent ectoparasites. *Med. Vet. Entomol.* 28, 314–318.
- Segura, F., Pons, I., Miret, J., Pla, J., Ortuño, A., Nogueras, M.M., 2014. The role of cats in the eco-epidemiology of spotted fever group diseases. *Parasits Vectors* 7, 353.
- Shih, C.M., Spielman, A., 1993. Accelerated transmission of Lyme disease spirochetes by partially fed vector ticks. *J. Clin. Microbiol.* 31, 2878–2881.
- Szabó, M.P., Rossi, G.F., Cabral, D.D., Martins, M.M., Gerardi, M., Amorim, M.P., Tsuruta, S.A., 2012. Experimental evaluation of birds as disseminators of the cosmopolitan tick *Rhipicephalus sanguineus* (Acari: Ixodidae). *Exp. Parasitol.* 132, 389–393.
- Thomas, J.E., Staubus, L., Goolsby, J.L., Reichard, M.V., 2016. Ectoparasites of free-roaming domestic cats in the central United States. *Vet. Parasitol.* 228, 17–22.
- Uspensky, I., 2009. Attachment of nymphal *Rhipicephalus sanguineus* (Acari: Ixodidae) to a human in an urban area followed by severe adverse reaction shortly before drop-off. *Folia Parasitol. (Praha)* 56, 67–69.
- Zakkyeh, T., Mohammad Ali, O., Nasibeh, H.V., Mohammad Reza, Y.E., Farhang, B., Fatemeh, M., 2012. First molecular detection of *Theileria ovis* in *Rhipicephalus sanguineus* tick in Iran. *Asian Pac. J. Trop. Med.* 5, 29–32.