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Ixodes ventalloi Gil Collado, 1936: A Vector Role to be Explored

Ana Sofia Santos and Maria Margarida Santos-Silva

Abstract

Ixodes Latreille, 1795 is the largest and broadest distributed genus of the family Ixodidae Murray, 1877. Its members are present in all zoogeographic regions, remote islands, and territories close to the poles. Plus, 63 species out of the 244 described have been recorded to feed on humans. Some are mega vectors, as those belonging to *Ixodes ricinus*-*I. persulcatus* complex, but others are so poorly studied that their vector role is difficult to access. This is the case of *Ixodes ventalloi* Gil Collado, 1936. This species is recorded in Northern Africa and Western Europe, mostly in Mediterranean basin countries, occurring along with other moisture-demanding ticks, as *Haemaphysalis* spp., *I. frontalis*, and *I. ricinus*. In fact, *I. ventalloi* not only shares vertebrate hosts (including humans) with the latter but may as well play a role in the enzootic cycle of some *Ixodes*-borne agents. This chapter updates information regarding this poorly studied tick, revising the available systematic, ecological, and microbiological data, discussing the potential public health relevance.

Keywords: *Ixodes ventalloi*, taxonomy, distribution, vertebrate hosts, tick-borne agents

1. Introduction

Ticks are a highly specialized group of obligate, bloodsucking, nonpermanent ectoparasitic arthropods of terrestrial vertebrates with a worldwide distribution. They present a hematophagic behavior in all active phases and parasitize mammals, birds, reptiles, amphibians, and occasionally man. Unique among Acari, ticks have a large body size being considered large mites with specialized mouthparts (hypostome) and specialized sensory structures on legs (tarsus I, Haller's organ) [1, 2]. These arthropods are among the most important vectors of human and animal disease. They are associated to the transmission of a great variety of pathogenic agents, including viruses, bacteria, and protozoa [3]. These pathogens are usually acquired by immatures, larvae, or nymphs, when ticks feed on infected hosts being maintained through their life and transmitted to naïve animals during the next blood meals, as nymphs and adults (horizontal transmission). Depending on the pathogen, ticks can also pass infection to the offspring (vertical transmission) or even to other ticks by feeding close to them (co-feeding). Ticks may also injure hosts without the involvement of infectious agents, just by the effects of salivary secretions, causing from a simple irritation to allergic reactions, toxicosis, and paralysis [4].

Among the family Ixodidae Murray 1877, the genus *Ixodes* Latreille 1795 is the largest, the broadest distributed, and one of the most important *taxon* regarding tick-borne diseases. It comprises a total number of 244 species of which 63 have been recorded to feed on humans [5]. Its members are present in all zoogeographic regions, remote islands, and territories close to the poles. In Europe and North Africa, the genus *Ixodes* is represented by 25 species [5]. Within this genus, several ticks may be considered mega vectors as those that belong to the *Ixodes ricinus*-*I. persulcatus* complex, but others are so poorly studied that their vector role is difficult to access. This is the case of *Ixodes ventalloi* Gil Collado, 1936. This is a species that is infrequently targeted in field trials and laboratory collections are scarce. In Portugal, an expansion of its distribution was observed, most likely as a collateral result of concerted efforts to increase knowledge on the subject [6–12]. The recent interest of other specialists has also generated updated descriptions of the morphological features relevant for diagnosis and the first molecular characterization of *I. ventalloi* populations with the analysis of its phylogenetic position in the group *Ixodes* [13, 14]. Regardless of this, information on the vector role of *I. ventalloi* remains challenging to access and poorly understood. This chapter intends to update information on *I. ventalloi* in order to call attention to this insufficiently studied tick, revising the available systematic, ecological, and microbiological data, discussing the potential public health relevance.

2. One species three names: a question of synonymy

I. ventalloi was first described by Gil Collado based on the morphological characters of a female tick parasitizing an *Athene noctua* captured in Barcellona [15]. The identification of this small-sized *Ixodes*, among other features, was based on the presence of particularly large and curved auriculae (**Figure 1**), differentiating it from the other European species, as the author wrote “Las aurículas de la base del capítulo, muy típicas y de forma de “asta de touro” (...) la distinguen netamente de las especies europeas (...)” [15]. In the following years of its description, *I. ventalloi* was misclassified and as a consequence, confusion arose regarding the morphological features and the ecology of this tick. *I. ventalloi* was either incorrectly ascribed as a new species, *I. thompsoni*, or confounded with *I. festai* and used for the redescription of the latter species mistaking both entities, as detailed by Gilot and Perez [16]. *I. festai*, originally described by Rondelli in 1926 based on the analysis of a female specimen found parasitizing a Libyan *Alectoris barbara*, is a bird-associated tick contrasting with the more permissive nature of *I. ventalloi*, as revised below in this chapter. Several works mention *I. thompsoni* and *I. festai sensu* Arthur as a synonym of *I. ventalloi*, but the definitive validation of this species was only achieved with the studies of Gilot, Morel and Perez [16–18].

Detailed descriptions of relevant morphological features have been subsequently updated, in some cases supported by illustrations and microscope images to better assist acarologists in *I. ventalloi* identification [14, 19–21]. Moreover, the application of mitochondrial DNA analysis using molecular targets, such as 12S rRNA, 16S rRNA, cytochrome *c* oxidase subunit 1 (*cox1*), has proven useful to complement the traditional morphological identification [12–14]. It also enabled the study of the population genetic structure. The molecular characterization of 92 *I. ventalloi* adults collected in cats from Lipari Island (Southern Italy) revealed the presence of a great genetic variability with the identification of eight haplotypes for 16S rRNA and 16 haplotypes for *cox1*, clustering in two sister clades—genogroup A, comprising 71% of the samples and genogroup B [13]. Interestingly, 16S rRNA sequences

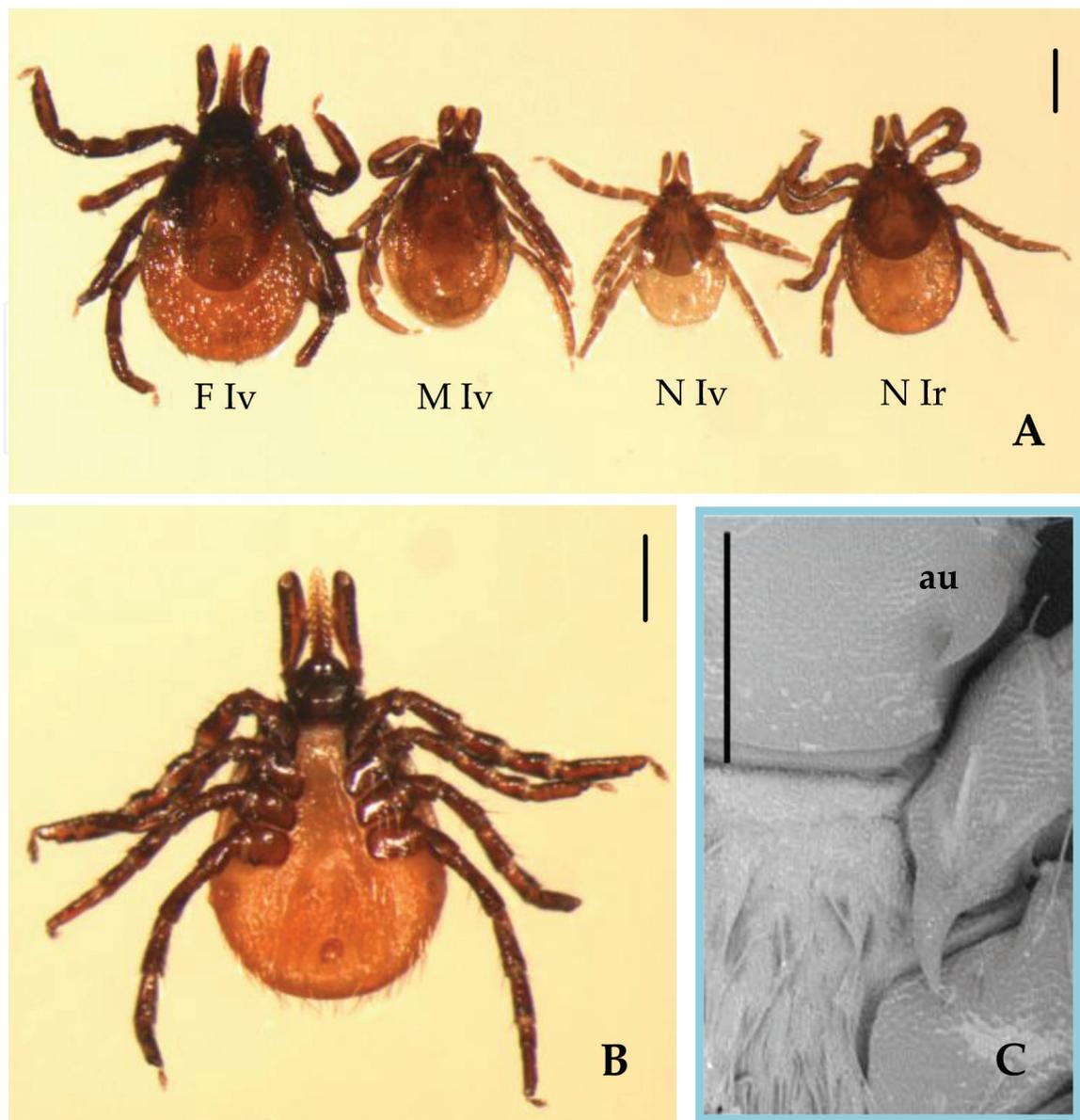


Figure 1. Morphological features of *Ixodes ventalloi*: (A) Size comparison of questing *I. ventalloi* female (F Iv), male (M Iv), and nymph (N Iv) with a questing *Ixodes ricinus* nymph (N Ir), dorsal view, scale bar 2 mm; (B) ventral view of a questing *I. ventalloi* female, scale bar 2 mm; (C) scanning electron microscopy detail of the recurved auriculae (au), the hallmark feature first pointed by Gill Collado [15] for differentiation from the other European *Ixodes*, scale bar 200 μ m.

closest to those belonging to genogroup A were also documented in Spain and Portugal [12, 14]. In the latter study, 12S and 16S rRNA genes have been targeted in 48 questing *I. ventalloi* (nymphs and adults), resulting in the identification of six haplotypes (IvH1-H6) but with a low degree of nucleotide variation placing them all in Latrofa's genogroup A [12]. **Figure 2** represents the phylogenetic distance, based on 16S rRNA sequences, of the *I. ventalloi* specimens collected in Italy, Spain, and Portugal, comparing to those related (>91% homology) *Ixodes* species. These results highlight the need for further genetic characterization of *I. ventalloi* population, increasing both the molecular coverage and the number of studied specimens from other geographical origins.

The *I. ventalloi* sequences obtained in the aforementioned studies were deposited in GenBank under the accession numbers: KU178964-KU178979 for *cox1*; KU178956-KU178963, KY231931, MF370642-43, MF621228, MF621233, MG210719-20 for 16S rDNA; MF370631-32, MF621221, MF621226, MG210717-18 for 12S rDNA.

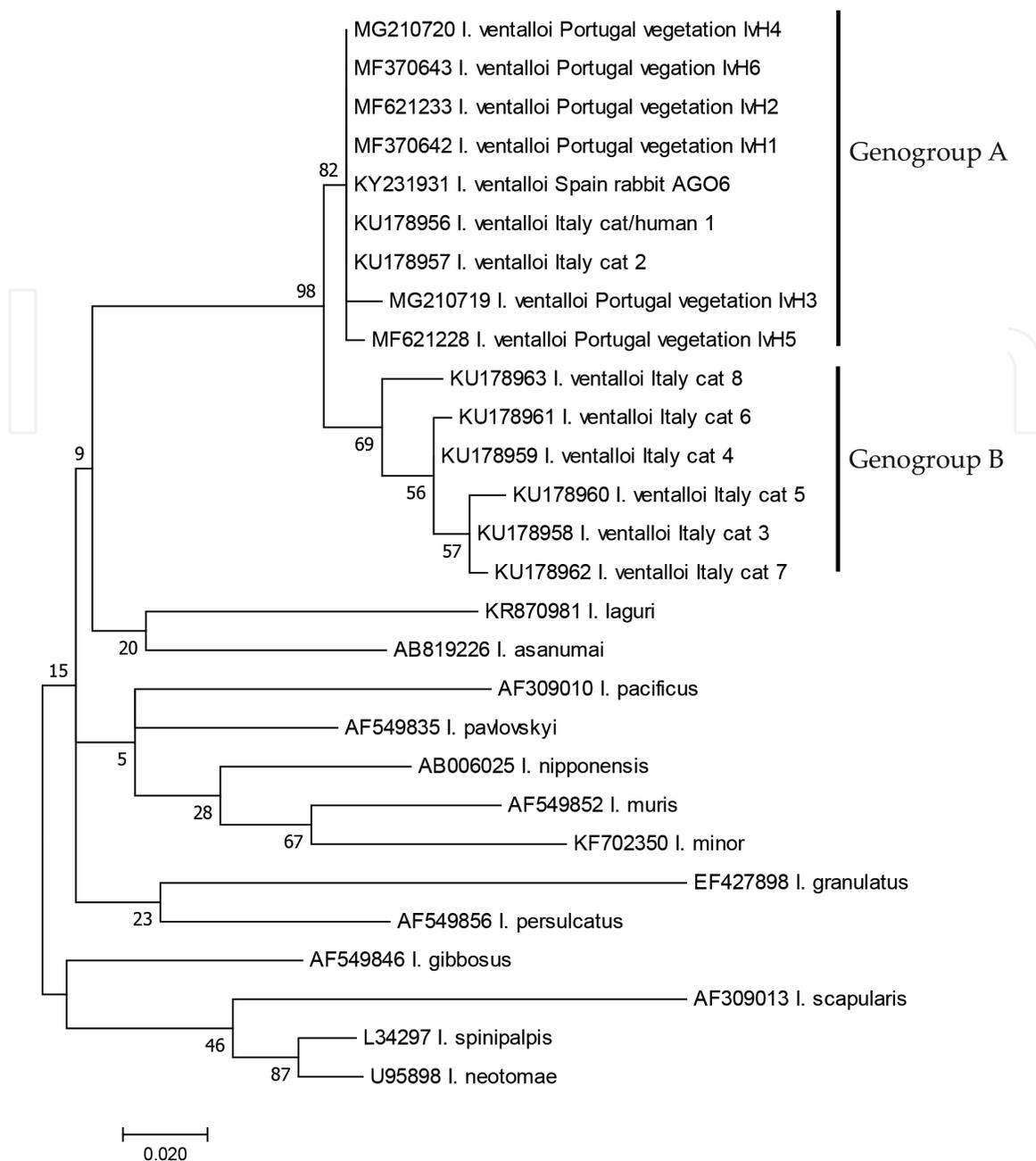


Figure 2. Phylogenetic trees based on 16S rRNA sequences obtained from *Ixodes ventalloi* collected in Italy, Spain and Portugal, comparing to sequences of other related (>91% homology) *Ixodes* species available in GenBank. Phylogenetic relationships were assessed computing the maximum likelihood method on MEGA7 [22]. Best fitting substitution models were determined using MEGA7 model selection method. Phylogenetic tree was constructed using General time reversible model, modulated by using a discrete Gamma distribution (+G) and based on the analysis of 1000 replicates. All positions with less than 75% site coverage were eliminated. That is, fewer than 25% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 247 positions in the final dataset. Accession numbers are followed by species name, and in some case the origin of sequences and haplotypes designation. Branch lengths represent the number of substitutions per site inferred according to the scale-bar.

3. The permissive “rabbit-tick”

The geographical distribution of *I. ventalloi* includes areas of western Mediterranean Europe (Portugal, Spain, Southern France, Central and Southern Italy, and Cyprus) and Northern Africa (Marroco and Tunisia) [14, 21]. This tick species was also documented in Great Britain (Channel Islands, Lundy Island, and Isles of Scilly) and in southwest Germany, probably as the result of introductions,

Host order and species	Tick stage/sex	Country (or region)	References
Birds			
Strigiformes			
<i>Asio flammeus</i>	F, M, N	Portugal	[9]
<i>Asio otus</i>	F	Great Britain	[16] ^a , [27]
<i>Athene noctua</i>	F	Spain,	[15]
<i>Tyto alba</i>	N	Portugal	[11]
Galliformes			
<i>Alectoris chukar</i>	ND	Cyprus	[31]
<i>Alectoris rufa</i>	F(+)	France, Italy	[16] ^b , [32]
<i>Phasianus colchicus</i>	F(+)	France, Italy, North Africa	[16] ^c , [32]
Passeriformes			
<i>Pica pica</i>	F	France	[16] ^b
<i>Turdus merula</i>	N	Great Britain, Portugal	[23, 33]
<i>Turdus pilaris</i>	F	France	[16] ^b
Gruiformes			
<i>Rallus aquaticus</i>	ND	Italy	[32]
Mammals			
Lagomorpha			
<i>Lepus europaeus</i>	F(+)	Cyprus, other European regions	[16] ^e , [31]
<i>Oryctolagus cuniculus</i>	F, M, N, L	France, Great Britain, Portugal, Spain, Morocco, North Africa	[7], [16] ^d [17, 18], [26] ⁱ , [23, 27–30]
Rodentia			
<i>Apodemus sylvaticus</i>	Im	Morocco	[26] ⁱ
<i>Eliomys quercinus</i>	F, N, L	Morocco, Portugal	[7], [26] ⁱ
<i>Gerbillus campestris</i>	Im	Morocco	[26] ⁱ
<i>Hystrix cristata</i>	ND	Italy	[34]
<i>Lemniscomys barbarus</i>	Im	Morocco	[26] ⁱ
<i>Mus spretus</i>	F, N, L	Morocco, Portugal	[7], [26] ⁱ
<i>Rattus rattus</i>	Im	Morocco	[26] ⁱ
<i>Rattus norvegicus</i>	N	Portugal	[11]
<i>Sciurus vulgaris</i>	F	Northern Africa	[16] ^f
Eulipotyphla			
<i>Crocidura russula</i>	N	Portugal	[7]
<i>Erinaceus europaeus</i>	F, M, N	Portugal, Spain and North Africa	[6], [16] ^f , [35]
Carnivora			
<i>Canis familiaris</i>	F	Portugal	[11]
<i>Felis catus</i>	F, M, N	France, Great Britain, Italy, Portugal, North Africa	[8, 10, 11, 13], [16] ^h , [27, 32, 36], [37]
<i>Genetta genetta</i>	F, M	Spain, other European regions	[16] ^g , [38]
<i>Herpestes ichneumon</i>	F, M	Spain	[38, 39]

Host order and species	Tick stage/sex	Country (or region)	References
<i>Lynx pardinus</i>	F, M	Spain	[38, 39]
Carnivora			
<i>Martes foina</i>	F	France	[16] ^b
<i>Meles meles</i>	F	France	[16] ^b
<i>Mustela nivalis</i>	F, M, N, L	Portugal	[7, 11]
<i>Mustela n. numidica</i>	Im	Morocco	[26] ⁱ
<i>Vulpes vulpes</i>	F, M, Im	Cyprus, Morocco Portugal, Spain	[6], [26] ⁱ , [31, 38–40]
Primata			
<i>Homo sapiens</i>	F(+)	France, Italy, Portugal	[16] ^b , [41–44]
Reptiles			
Squamata			
<i>Agama impalearis</i>	Im	Morocco	[26] ⁱ
<i>Chalcides polylepis</i>	Im	Morocco	[26] ⁱ
<i>Eumeces algeriensis</i>	Im	Morocco	[26] ⁱ
<i>Psammotromus algirus</i>	Im	Morocco	[26] ⁱ

ND—No detail is provided regarding sex/stage of the collected tick(s); Im—Immature(s) stage(s) not detailed; F—Female(s); F(+)—Female(s) and possible other specimens as information regarding sex/stage is not detailed in all references; M—Male(s); N—Nymph(s); L—Larva(e).

^{a–h}In Gilot and Perez [16], the country or geographical regions was deduced based on the authors' descriptions of the origin of *I. ventalloi* specimens. ^aThompson collection. ^bGilot collection. ^cGilot and Morel collection. ^dGilot, Morel, Institute Pasteur and Clifford collections. ^eInstitute Pasteur collection. ^fMorel collection. ^gNeumann collection. ^hGilot, Morel and Thompson collections.

ⁱBailly-Choumara et al. [26] list of the Moroccan host is used with reservations since it was published prior to the differentiation of *I. ventalloi* from *I. festai*. In any case, the authors were aware about the synonymy of *I. ventalloi* and *I. festai sensu Arthur*. Moreover, *I. festai* is also listed but placed apart from *I. ventalloi*.

Table 1.
List of vertebrate species found with *Ixodes ventalloi* ticks.

but the establishment of *I. ventalloi* populations was only confirmed in the Britain islands [23, 24]. In Portugal, *I. ventalloi* was first identified in 1985, and since then, it has been described across the country mainly in littoral mainland areas and along with other moisture-demanding ticks, such as *Haemaphysalis* spp., *Ixodes frontalis*, *I. ricinus* [6–8, 10–12, 25].

I. ventalloi is regarded as a three-host, endophilic, and monotropic tick. All development stages are commonly found parasitizing *Oryctolagus cuniculus* and associated to lagomorph's environment; thus, it is popularly designated as the “rabbit-tick” [7, 16–18, 23, 25–30]. However, the list of vertebrate hosts parasitized by *I. ventalloi* is much more broader, including several species of rodents and other small mammals, medium-size carnivores, and occasionally ground-dwelling birds and birds of prey [6–11, 13, 15, 16, 23, 26, 27, 31–43]. **Table 1** resumes the host species that have been documented with *I. ventalloi* ticks. Close to 40 species are so far listed revealing the permissive feeding behavior of this tick, which is not restricted to wild animals. In fact, *I. ventalloi* were found feeding on humans and companion animals, mostly cats and sporadically dogs, as shown in **Table 1**. Regarding cats, the first record of this tick-host association date back to the description of *I. thompsoni* (synonym of *I. ventalloi*) from the material collected in Lundy Island by Thompson [16]. Since then, *I. ventalloi* has been recurrently found feeding on cats in almost all

areas where it occurs and in some cases described as the predominant tick species found on this host [8, 10, 11, 13, 27, 32, 36, 37]. Cat parasitism by *I. ventalloi* might be explained by the host free-roaming and hunting habits that place them in close contact with the ground and low vegetation when ambushing small animals. Although *I. ventalloi* is regarded as having a limited potential for dispersal, it can be found actively seeking for hosts at the ground level. This was proven by us in previous studies, when dragging vegetation and grassy ground resulted in the collection of 175 questing *I. ventalloi*, including nymphs, males and females [8–12]. The same result was recently obtained by Torina et al. [45] that have collected 1425 questing *I. ventalloi*, including all tick stages, by dragging vegetation in Palermo's areas during a 2-year study.

The particular association of cats to *I. ventalloi* may contribute to bring this tick to domestic environments and to promote human exposure. In fact, *I. ventalloi* has long been listed as a human-biting tick [16, 41]. In Portugal, the authors recorded the first case of human parasitism by this species in 2014, on behalf of the Surveillance Network for Vectors and Vector-borne Diseases (REVIVE), and keep documenting it every year since then [44, 46]. Although *I. ventalloi* represents a small percentage (less than 1%) of the species found feeding on humans, it is under the scope for potential infections by human pathogens [44, 46]. The increasing number of agents associated to this species in recent years brought back the question regarding the *I. ventalloi* public health relevance.

4. A vector's potential neglected or negligible?

During several years, information regarding *I. ventalloi* pathobiome was almost absent. The first association of this tick to a potential tick-borne agent was reported in 1984 by Chastel et al. [47]. In this study, strains of the coltivirus Eyach (EYAV), a virus belonging to Colorado tick fever group, were isolated from both *I. ventalloi* and *I. ricinus* ticks that were found parasitizing a wild rabbit in Northwestern France. Eyach virus was previously described in *I. ricinus* from West Germany, subsequently found on several wild mammals and indirectly linked to patients with neurological disorders, as tick-borne encephalitis, polyradiculoneuritis, and meningopolyneuritis, on a base of serology [48]. In 2004, we have also reported *I. ventalloi* infection by *Anaplasma phagocytophilum*, a species with variant strains implicated in human and domestic animal cases of granulocytic anaplasmosis [8]. The growing interest on *I. ventalloi* observed in the last 10 years has resulted in an increasing number of papers and the detection of diverse microorganisms associated to this tick species. **Table 2** resumes the microbial agents that have been found in *I. ventalloi*, providing information on ticks stage, sex, and molecular identification (haplotypes), when available. Overall, 13 agents have already been associated to *I. ventalloi*, and infected ticks were found feeding on wild animals, as well as on domestic cats and on a human, pointing for a potential role as vector that might have both medical and veterinary implications.

Another justification for the presence of agent's nucleic acids in parasitizing ticks can also be the presence of host-infected blood in arthropods' midgut rather than a true vector potential. However, it is worthy of note that some of the *I. ventalloi* positives were indeed unfed ticks, as detailed. The first record dates back to 2004 when the authors were investigating *A. phagocytophilum* in *I. ricinus* and their sympatric ticks in Setubal District, Portugal [8]. The screened sites were mainly suburban wooded areas in some cases used for grazing and with evidence

Microorganism	Ticks origin	Ticks haplotypes§	Reference
Virus			
Coltivirus Eyach	<i>Oryctolagus cuniculus</i>	ND	[47]
Bacteria			
<i>Anaplasma marginale</i>	Vegetation	ND	[49]
<i>A. phagocytophilum</i>	Vegetation, <i>Felis catus</i>	ND	[8, 10]
	Vegetation	IvH1, IvH3, IvH5	[12]
<i>Ehrlichia canis</i>	<i>Felis catus</i>	ND	[36]
<i>Ca Neohrlichia</i> sp.	Vegetation	ND	[12]
<i>Rickettsia helvetica</i>	<i>Asio flammeus</i>	ND	[9]
	<i>Lynx pardinus</i> , <i>Vulpes vulpes</i>	ND	[38]
	<i>Felis catus</i> , <i>Rallus aquaticus</i>	ND	[32]
	<i>Felis catus</i> *	ND	[36]
	<i>Homo sapiens</i>	Haplotype 1	[13, 37]
<i>R. monacensis</i>	<i>Oryctolagus cuniculus</i>	ND	[29]
	<i>Lynx pardinus</i> , <i>Vulpes vulpes</i> , <i>Genetta genetta</i>	ND	[38]
	<i>Felis catus</i> *	ND	[32, 36]
<i>Coxiella burnetii</i>	<i>Alectoris chukar</i> , <i>Lepus europaeus</i>	ND	[31]
	Vegetation	IvH2, IvH3, IvH5, IvH6	[12]
<i>Bartonella clarridgeiae</i>	<i>Felis catus</i> ‡	ND	[36]
<i>Borrelia valaisiana</i>	<i>Felis catus</i> , <i>Rallus aquaticus</i> , <i>Phasianus colchicus</i> ¥	ND	[32]
<i>B. spielmanii</i>	<i>Felis catus</i> , <i>Phasianus colchicus</i> ¥	ND	[32]
Protozoa			
<i>Leishmania infantum</i>	<i>Felis catus</i> ‡	ND	[36]
<i>Theileria annulata</i>	Vegetation	ND	[49]

*One tick co-infected with *Rickettsia helvetica* and *R. monacensis* [36].

‡One female tick coinfecting with *Bartonella clarridgeiae* and *Leishmania infantum*.

¥One tick co-infected with *Borrelia valaisiana* and *B. spielmanii*.

§All these haplotypes have been identified as belonging to genogroup A and were submitted to Genbank under the accession numbers: Haplotype 1, KU178956 (16S rDNA) and KU178964 (cox1); IvH1, MF370631 (12S rDNA) and MF370642 (16S rDNA); IvH2, MF621226 (12S rDNA) and MF621233 (16S rDNA); IvH3, MG210717 (12S rDNA) and MG210719 (16S rDNA); IvH5, MF621221 (12S rDNA) and MF621228 (16S rDNA); IvH6, MF370632 (12S rDNA) and MF370643 (16S rDNA), as previously described [12, 13].

Table 2.
List of microorganisms and parasites found in *Ixodes ventalloi*.

of wild animals presence, as rabbits. Out of the 93 *I. ventalloi* collected, *A. phagocytophilum* was recorded in a questing nymph and also in a male found attached to a free-roaming cat. The sequences obtained from both ticks were found to be a new *A. phagocytophilum* variant based on *groEL* and *msp2* gene analysis [8]. This finding was subsequently reinforced by the detection of the same *A. phagocytophilum* variant also in an *I. ventalloi* female feeding on a cat from another district of mainland, Santarém District [10]. More recently, a retrospective study using the DNA material stored from Setúbal district ticks also resulted in the detection

of questing *I. ventalloi* specimens of *Anaplasma marginale* (four nymphs and one male) and *Theileria annulata* (one female) [49]. New data that link more agents to questing *I. ventalloi* were submitted for publication in the beginning of 2018 [12]. That study was undertaken in Parque Florestal de Monsanto (PFM), a recreational area located in the urban perimeter of Lisbon city. This is a highly used park for petting and several outdoor activities. Overall, eight tick species were found questing in PFM with a preponderance of *I. ventalloi*. A preliminary 1-year screening to define the best season for collection, established that the period of activity for this species extended from November to June, with a peak in spring (Figure 3) [50]. Interesting, both of these findings (abundance and seasonality) were reinforced in Torina et al. comprehensive study [45]. In our case, the diversity of PFM ticks and the particular abundance of *I. ventalloi* were attributed to the park's wild population, composed of over 100 species of small mammals and birds [51].

Regarding tick-borne agents, questing *I. ventalloi* in PFM were found harboring *A. phagocytophilum* (two males, one female, and two nymphs), *Coxiella burnetii* (five males, three females, and one nymph), and a potentially new agent

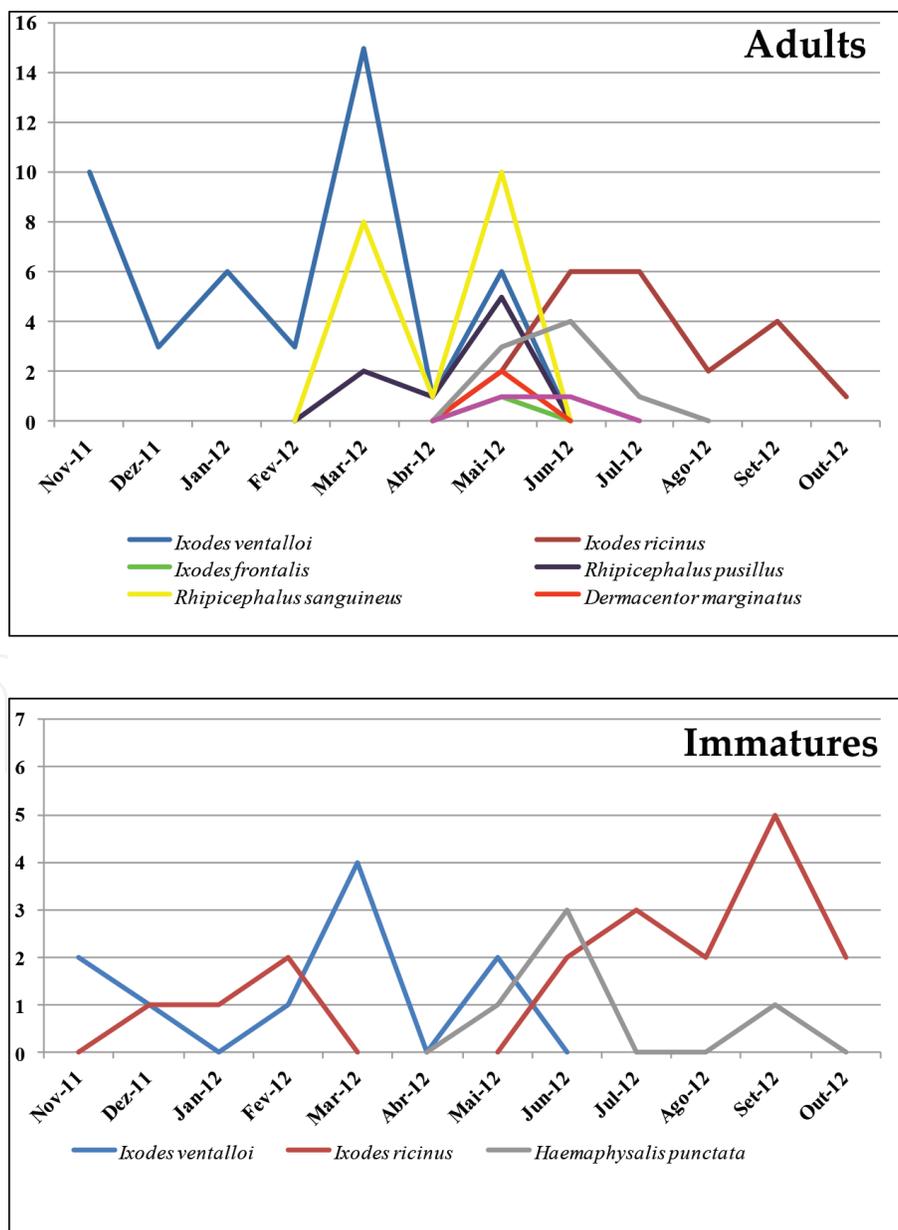


Figure 3. The distribution of immature and adult tick species collected in PFM during 1-year period (2011–2012) [50].

close related to *Candidatus* (*Ca.*) *Neoehrlichia mikurensis* (one female and one male). Interestingly, two *A. phagocytophilum* variants were detected [12]. The more representative was a new variant of *A. phagocytophilum* previously detected in both Setúbal and Santarém districts (and here found on four ticks) [8, 10]. This reinforces previous data sustaining a divergent variant of *A. phagocytophilum*, not clustering in none of the four ecotypes defined by Jahfari et al. [52], with the closest sequence sharing only 95% homology and belonging to ecotype IV that is composed of sequences of the agent derived from birds. Another *A. phagocytophilum* variant was obtained from a single *I. ventalloi*, clustering the ecotype I that is composed by agent's sequences associated to human and domestic animal cases of granulocytic anaplasmosis [52]. It was also worth of mention that positive ticks were found questing in different occasions showing the existence of active cycles for these agents in PFM [12]. The molecular identification of nine positive ticks confirmed that all belonged to *Latrofa*'s genotype A, based on 16S rDNA analysis. The obtained haplotypes and the GenBank accession numbers are presented in **Table 2**.

In all the aforementioned Portuguese areas, infected *I. ventalloi* were found questing along with other moisture-demanding ticks, as the mega-vector *I. ricinus*. Both tick species are considered sympatric sharing geographical distribution, vertebrate hosts, and possible their agents [11]. The presence of alternate ticks (generally endophilic ticks) has been associated to the existence of secondary maintenance cycles for some *Ixodes*-borne agents [53]. If *I. ventalloi* has such a role and thus contributes indirectly to the occurrence of *I. ricinus*-borne diseases is yet to be investigated.

5. Conclusion

I. ventalloi has been relegated to the sidelines for years due to its endophilic nature, apparent host specificity, and unknown vector importance. Accumulated evidence is, however, revealing a different reality for this small *Ixodes*. As revised here, *I. ventalloi* presents a permissive feeding behaviors that might promote exposure to several blood-borne pathogens and the list of agents found in this species keeps growing. Of note is the fact that *I. ventalloi* is broadly found feeding on cats and can also parasitize men. Moreover, it is sympatric to the mega-vector *I. ricinus* and might contribute to the maintenance of its agents. Altogether these suggest a vector role for *I. ventalloi*, either directly or by sympatry with other ticks species, with potential public health implications and deserving further investigation.

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Conflict of interest

The authors do not disclose any conflict of interest.

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