



# Vectors and Vector Borne Diseases: Morphological and Molecular Diagnosis, Risk Assessment, Population Genetics and Control Strategies

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**Abstract.** Vector-borne diseases are transmitted through arthropods and new associations between them and pathogens are continually being described. In the present study, authors briefly address their research areas, reporting multiple collaboration studies and major findings achieved by the CIISA's Parasitology and Parasitic Diseases team and its partners over the past few years. Starting with *Culicoides*, their importance as vectors of animal/human diseases, the description of new species and also species modelling as disease surveillance is discussed. Studies on national lice species in wild birds raise awareness to a so far neglected group, evincing known records and new contributions towards a better knowledge. Tick and tick-borne pathogen studies over the years have contributed to a national portrait of species geographic and seasonal distribution and to pathogen endemic associations, describing most common public and veterinary health threats. In this regard, morphology identification strategies are enounced and molecular markers used considered. Due to a reported rise in prevalence, piroplasms impact is

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M. Santos-Silva---In memoriam.

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particularly focused as a major concern for cattle production industry sector. A national overview concerning *Theileria* and *Babesia* prevalence inferences and diagnosis specificities are reviewed. Ultimately, a national *Dirofilaria immitis* and *Angiostrongylus vasorum* epidemiological update is given while diagnosis and treatment options are debated.

**Keywords:** VBDs · Diagnosis · Risk-assessment · Control · Portugal

**Vector-borne diseases** are raising awareness throughout the globe due to their devastating repercussions in human and animal health. Known modern challenges as environment, socio-economy and governmental policies are by themselves changing the distribution and the impact of such diseases. Prevention and disease control are dependent on distribution and abundance as well as on population dynamics knowledge. At CIISA's Parasitology and Parasitic Diseases Laboratory (LPDP) different studies are integrated in multidisciplinary studies that continuously contribute to a national overview of the most important vectors and pathogens of animal and also human concern.

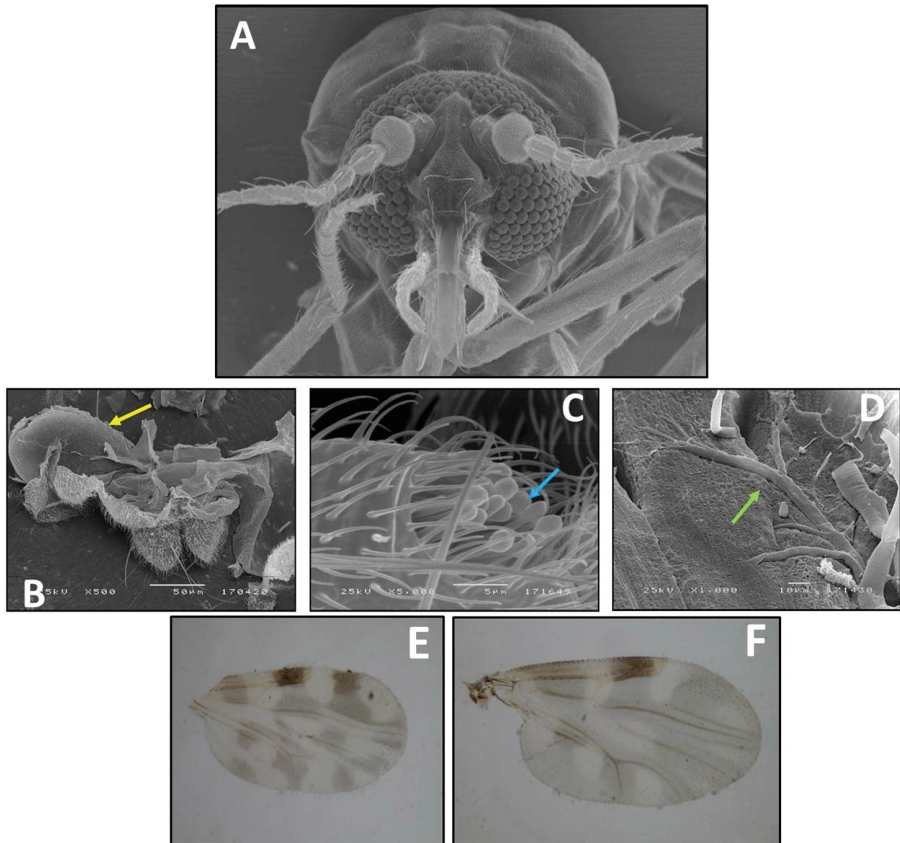
**Culicoides Midges in Portugal: What's New in the Last Decade?** *Culicoides* (Diptera: Ceratopogonidae) genus contains important vectors of animal and human diseases including Bluetongue Disease (BTD), African Horse Sickness, Schmallenberg and filariasis (Linley *et al.* 1983; Mellor *et al.* 1990; Balenghien *et al.* 2014). The species *Culicoides imicola* is the main vector of BTD in Southern Europe and Africa (Wilson and Mellor 2009). Before 1998, BTD occurred sporadically in Europe (Barros *et al.* 2007; Wilson and Mellor 2009). However, since 1998 the scenario changed drastically, with the disease affecting several Southern European countries (1998–2005) and also Central and Northern Europe between 2006–08 (Wilson and Mellor 2009; Stenberg Lewerin *et al.* 2010). The occurrence of the disease in regions where *Culicoides imicola* was absent as in Central and Northern Europe is explained by the presence of Obsoletus group species and *C. pulicaris*, which gained the capacity to acquire, maintain and transmit Bluetongue virus (BTV) to susceptible hosts (Vanbinst *et al.* 2009).

The first incursion of BTV in mainland Portugal was reported in July 1956 (Barros *et al.* 2007). In 4 years, around 180,000 sheep were killed due to BTV serotype 10 (BTV-10) (Barros *et al.* 2007; Office International des Epizooties 2018). In 1960, the country was declared free of BTV (Barros *et al.* 2007). A new introduction of BTV (BTV-4) in mainland Portugal was registered in November 2004, after a 44-year period of epizootic silence. BTV-1 outbreaks have occurred several times in the last decade in mainland Portugal (2007–12 and 2015–17), as well as BTV-4 (2004–06, 2013 and 2018) (Ramilo *et al.* 2018).

Between 2005–2013, the Portuguese National Authority for Animal Health (DGAV) in partnership with the Faculty of Veterinary Medicine established a National Entomologic Surveillance Program (NESP) for BTD in recognition of the high and continuing threat to the Portuguese livestock sector from *Culicoides*-borne viruses (Ramilo *et al.* 2012). NESP was created to better perceive the distribution of different *Culicoides* species, directly reporting to the DGAV the presence of BTD vectors, allowing them to act in real time and implement the necessary measures to prevent the spread of the disease in case of an outbreak. NESP covered all Portuguese regions, including mainland

Portugal, Azores and Madeira archipelagos (Ramilo *et al.* 2012; Ribeiro *et al.* 2015). The NESP identified for the first time in mainland Portugal and Azores archipelago a total of 23 *Culicoides* species including the description of a new species, *Culicoides paradoxalis* Ramilo and Delécolle 2013 (Ramilo *et al.* 2013, 2018) (Fig. 1). The new species identification, resulted from a collaboration with the Faculty of Sciences of the University of Lisbon (FCUL), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France) and the University of Strasbourg, France.

After 2013, several works have been performed with other institutions (CIRAD, University of Strasbourg and FCUL) in order to identify *Culicoides* specimens using morphological and molecular biology techniques and to better understand *Culicoides imicola* distribution and its geographic origin (Jacquet *et al.* 2015, 2016). Furthermore, our group participated in VECTORNET Project – *Culicoides* group (2014–18) capturing



**Fig. 1.** A - *C. paradoxalis* a new species for science; B – *C. circumscriptus* spermatheca (yellow arrow); C – Sensorial organs from the 3<sup>rd</sup> palpus segment of *C. obsoletus* (blue arrow); D – Vessels that supply thorax muscles with nutrients (green arrows); E – Wing pattern of *C. imicola*; F – Wing pattern of Obsoletus group species.

*Culicoides* near horse farms comparing species caught with those captured near domestic ruminants (Ramilo 2016); identifying *Culicoides* species from different European countries (Switzerland, Iceland, Latvia, Denmark and Norway) and providing laboratory training to international researchers who needed to identify *Culicoides* specimens captured in their country. Such works highlight the importance of this field of study and the international recognition of the work developed at our group by our partners.

In 2016, our group organized the E-SOVE Congress, related with vector and vector-borne diseases. It was held at the Faculty of Veterinary Medicine, University of Lisbon, and brought together several international teams of different research areas, including those working with *Culicoides*. During this congress, a training course concerning *Culicoides* capture and morphological identification was attended by international researchers featuring LPDP team capacity to deliver highly specific training to peers within this research area.

With all the data collected during NESP, our team produced a PhD thesis (Ramilo 2016), a book chapter (Alexandre-Pires *et al.* 2010), a revision article (Ramilo *et al.* 2018), several scientific articles, some of them in collaboration with international teams (Ramilo *et al.* 2012, 2013, 2017; Ribeiro *et al.* 2015; Jacquet *et al.* 2015, 2016), 22 oral and 15 poster presentations. Furthermore, an identification key for *Culicoides* species present in Portugal is also provided online for researchers and students interested in this field of study (<http://www.fmv.ulisboa.pt/uploads/2017/11/5a0ac47a19090.pdf>).

Presently, several works are being developed at our laboratory concerning biting midges: traps are being placed near wild animals in a zoological context to know *Culicoides* fauna present; identification studies of *Obsoletus* group specimens with intermediate morphological characteristics (between *C. obsoletus* and *C. scoticus* species) to assert their phylogenetic position; characterizing the distribution of *Obsoletus* group species in mainland Portugal; capture of live *Culicoides* specimens to evaluate the wing interference pattern of different species; audio recording of *Culicoides* wing movement to evaluate differences between species and identification of *Culicoides* aberrant structures to proceed with correct identification of *Culicoides* species even in the presence of these anatomical modifications.

**Overview of Chewing Lice of Wild Birds in Portugal: A Neglected Group of Ectoparasites.** Chewing lice or Mallophaga, are the most common ectoparasites found in birds. Avian chewing lice belong to one of two suborders, Amblycera or Ischnocera, being the latter the most representative (Price *et al.* 2003). Morphologically, chewing lice are characterized by a small size ( $\leq 0,8$ –11 mm long), a segmented body dorsoventrally flattened and segmented antennae (3–5 segments). All chewing lice of birds are obligate and permanent ectoparasites, feeding mainly on feathers and skin with the entire life cycle occurring on hosts (Clayton *et al.* 2008). Some species of Amblycera suborder include blood in their diet by scratching or nibbling at the soft skin at the base of the host feathers (Ash 1960). Physical contact between birds is considered the major route of transmission of Mallophaga species. So, being more effective between copulating birds and between parents and their offspring (Hillgarth 1996; Tompkins *et al.* 1996). According to Keirans (1975) ischnoceran species may be transported by phoresis on hippoboscids flies. Generally, chewing lice are highly specific to their hosts at a genus or species level (Clayton *et al.* 2008). However, some mallophagan species

are less specific, and according to Clayton *et al.* (2004) that can be partly explained by the non-host specificity of hippoboscids flies.

In Portugal, the number of louse species documented from wild birds is limited. The first document concerning endemic chewing lice of wild birds was published by Tendeiro (1962), with a monographic review of the genus *Columbicola* Ewing, 1929 collected not only in Portugal, but worldwide. More recently, Literák *et al.* (2015), focusing on chewing lice parasitizing blackcaps (*Sylvia atricapilla*) in the Azores, recorded two new chewing louse species in Portugal: *Brueelia tovornikae* (Balát 1981), currently named as *Guinaraesiella tovornikae* (Balát 1981) and *Myrsidea sylviae* Sychra and Literak, 2008. Tomás *et al.* (2016) published a more complete study of chewing lice in Portugal. These authors sampled wild birds of different genus admitted at the Wildlife Rehabilitation and Investigation Centre of Ria Formosa –ALDEIA Association (RIAS/ALDEIA) and also from mist-nets captured animals during scientific ringing sessions performed at the Ria Formosa Natural Park. This work registered 18 louse species for the first time in Portugal, including a nymph of the genus *Strigiphilus* Mjöberg, 1910 collected from a Eurasian Eagle-Owl (*Bubo bubo*) (Table 1).

When present in small numbers a variety of behavior defenses, with preening as the most important, helps hosts to tolerate infestation with no apparent effects. Large infestations, however can cause dermatitis and pruritus leading to feather and skin damage (Johnson and Clayton 2003; Clayton *et al.* 2008). According to Booth *et al.* (1993), the dramatic increase of lice feeding on feathers reduces the abdominal plumage, increasing thermal conductance and respective metabolic rates required to maintain body temperature. The maintenance of elevated metabolic rates induces birds to use their fat reserves causing a chronic decline in body mass. In the specific case of the louse *Machaerilaemus malleus* (Burm 1838), Kose *et al.* (1999) showed that Barn Swallows (*Hirundo rustica*) males with large white tail spots without parasite damage are selected by females for displaying reliable quality feathers. Furthermore, it is believed that the holes caused by the parasites in the spots may increase feathers breakage and permeability, changing the aerodynamic efficiency of the flight, as occurs with birds parasitized by mites (Bonser 2001).

Chewing lice can have effects at population level, as recorded by Samuel *et al.* (1982). The authors reported severe hemorrhagic ulcerative stomatitis of juvenile American White Pelicans (*Pelecanus erythrorhynchus*), parasitized by amblycera *Piagetiella peralis* (Leidy 1878) and some birds were found dead. However, it is not clear whether lice were the cause of death. Historically, it has been thought that chewing lice fed very occasionally on host blood, i.e., they were ignored as potential vectors or intermediate hosts of other parasites. However, a better knowledge of Mallophaga feeding habits allowed to understand their indirect effects on hosts, as a detailed reviewed by Saxena *et al.* (1985) has shown. For example, the amblyceran *Pseudomenopon pilosum*, common lice of Rallidae birds, already identified in Portugal by Tomás *et al.* (2016), transmits the filarid *Pelecitus fulicaeatrae* (Diesing 1861) to *Fulica americana* (Bartlett and Anderson 1987). The authors found adult worms among tendons at the ankle joint and microfilariae in the skin of the feathered portions of the legs of infected birds, as well as microfilariae, first-, second- and third-stage of this nematode in abdomen of lice, suggesting that *P. pilosum* transmits the filarial *P. fulicaeatrae* when the louse takes

**Table 1.** Reports of chewing lice species in wild avian hosts from Portugal.

Avian host	Louse species	References
<b>ACCIPITRIFORMES</b>		
Bonelli's Eagle ( <i>Aquila fasciata</i> )	<i>Degeeriella fulva</i> (Giebel 1874)	Tomás et al. 2016
Common Buzzard ( <i>Buteo buteo</i> )	<i>Degeeriella fulva</i> (Giebel 1874)	Tomás et al. 2016
	<i>Craspedorrhynchus platystomus</i> (Burm. 1838)	Tomás et al. 2016
	<i>Laemobothrion (L.) maximum</i> (Scopoli 1763)	Tomás et al. 2016
Eurasian Griffon ( <i>Gyps fulvus</i> )	<i>Falcolipeurus quadripustulatus</i> (Burm. 1838)	Tomás et al. 2016
	<i>Laemobothrion (L.) vulturis</i> (Fabricius [J.C.] 1775)	Tomás et al. 2016
	<i>Colpocephalum turbinatum</i> Denny 1842	Tomás et al. 2016
	<i>Nosopon casteli</i> Tendeiro 1959	Tomás et al. 2016
<b>ANSERIFORMES</b>		
Common Teal ( <i>Anas crecca</i> )	<i>Trinoton querquedulae</i> (L., 1758)	Tomás et al. 2016
<b>CHARADRIIFORMES</b>		
Yellow-legged Gull ( <i>Larus michahellis</i> )	<i>Actornithophilus piceus lari</i> (Packard 1870)	Tomás et al. 2016
Dunlin ( <i>Calidris alpina</i> )	<i>Actornithophilus umbrinus</i> (Burm. 1838)	Tomás et al. 2016
	<i>Luniceps schismatus</i> Gustafsson and Olsson 2012	Tomás et al. 2016
	<i>Austromenopon lutescens</i> (Burm. 1838)	Tomás et al. 2016
<b>COLUMBIFORMES</b>		
Common Wood-Pigeon ( <i>Columba palumbus</i> )	<i>Columbicola claviformis</i> (Denny 1842)	Tendeiro 1962
Rock Pigeon ( <i>Columba livia</i> )	<i>Columbicola columbae</i> (L., 1758)	Tendeiro 1962
<b>GRUIFORMES</b>		
Common Coot ( <i>Fulica atra</i> )	<i>Pseudomenopon pilosum</i> (Scopoli 1763)	Tomás et al. 2016

(continued)

**Table 1.** (continued)

Avian host	Louse species	References
<b>PASSERIFORMES</b>		
Blackcap ( <i>Sylvia atricapilla</i> )	<i>Guinaraesiella tovornikae</i> (Balát 1981)	Literák <i>et al.</i> 2015
	<i>Myrsidea sylviae</i> Sychra and Literak, 2008	Literák <i>et al.</i> 2015
<b>PHOENICOPTERIFORMES</b>		
Greater Flamingo ( <i>Phoenicopterus roseus</i> )	<i>Colpocephalum heterosoma</i> Piaget 1880	Tomás <i>et al.</i> 2016
	<i>Trinoton femoratum</i> Piaget 1880	Tomás <i>et al.</i> 2016
<b>STRIGIFORMES</b>		
Eurasian Eagle-Owl ( <i>Bubo bubo</i> )	<i>Strigiphilus</i> sp. Mjöberg 1910	Tomás <i>et al.</i> 2016
<b>SULIFORMES</b>		
Northern Gannet ( <i>Morus bassanus</i> )	<i>Pectinopygus bassani</i> (Fabricius [O.] 1780)	Tomás <i>et al.</i> 2016
	<i>Eidmanniella pustulosa</i> (Nitzsch [In Giebel] 1866)	Tomás <i>et al.</i> 2016

a skin feeding. Mallophagan vectors are not only associated with skin microfilariae. Seegar *et al.* (1976) reported that Tundra swan (*Cygnus columbianus*) is infected by common heartworm, *Sarconema eurycera* Wehr 1939, when amblyceran louse *Trinoton anserium* (Fabricius [J.C.] 1805) takes a bloodmeal. On the other hand, ischnoceran lice were associated with *Eulimdana* species, filarid nematodes of the Charadriiformes neck. According to Bartlett (1993) Mallophaga species, such as *Carduiceps clayae* Timmer 1954 and *Luniceps numenii numenii* (Denny 1842), act as intermediate host and transmit these worms when they are ingested during bird preening. Bacteria and viruses have also been isolated experimentally from chewing lice as summarized by Saxena *et al.* (1985). For example, the virulent bacteria *Pasteurella multocida*, the pathogen responsible for epidemic outbreaks of fowl cholera, were found in the digestive tract and feces of *Melopon gallinae* (L., 1758) and *Menacanthus stramineus* (Nitzsch 1818) (previously named as *Eomenacanthus stramineus*) (Derylo 1970). The author suggested “transmission was thought to be due to direct contamination of a wound with feces or by ingestion of the infected louse” by the Red Junglefowl (*Gallus gallus*). In the case of the role played by mallophagans in viruses transmission, Howitt *et al.* (1948) isolated the Eastern equine Encephalitis virus from fowl lice *Menacanthus stramineus*, suggesting that these and other species of chewing lice, with blood feeding habits, may be important vectors of the virus among birds, especially in the absence of other vectors. However, these authors’ assumptions about the lice role in transmission of viruses and bacteria are not totally clear.

**Ticks and Tick-Borne Disease with National Relevance.** Ticks are ectoparasites that feed exclusively on blood, parasitizing virtually all terrestrial and semi-aquatic vertebrates across the zoogeographic regions of the world. Three living tick families are described: Argasidae, Nutalliellidae and Ixodidae. The latter is the most studied and has the greater number of species with veterinary and medical impact. In Portugal, the last comprehensive revision of the family Ixodidae Murray 1877, dates from 2011 including all the available information gathered on nationwide but also more directed surveys (Santos-Silva *et al.* 2011). Subsequent publications have contributed to further increase the knowledge of the Portuguese tick fauna (Norte *et al.* 2012; Estrada-Peña *et al.* 2014). Presently, it is recognized the occurrence of 21 species of ixodid ticks, distributed by five genera (Table 2). Some of these ticks are host specific, mostly associated to wild animals and generally poorly studied, as is the case of *Haemaphysalis hispanica*, *H. inermis*, *Ixodes acuminatus*, *I. frontalis*, *I. simplex*, *I. vespertilionis* (Santos-Silva *et al.* 2011). Other species were more recently described in Portugal and their impact as pests or vectors is still unknown, such as *Ixodes arboricola* and *I. inopinatus* (Norte *et al.* 2012; Estrada-Peña *et al.* 2014). In any case, the majority of the tick species occurring in our country are commonly found feeding on livestock, companion animals and humans, having a considerable veterinary and public health impact. They can cause anemia due to blood depletion observed in high-rates of tick parasitism, skin secondary infections and injury, anaphylactic and toxic reactions against arthropod's saliva, but most of all, ticks are responsible for the inoculation of pathogenic agents. During the years, several tick-borne agents have been described in questing ticks collected across the country suggesting a potential vector role for some Portuguese species. Table 2 lists the agents that have been detected in questing ticks in Portugal. Most of these agents are bacteria exclusively vectored by ixodid ticks and belonging to Spotted Fever Group *Rickettsia*, *Borrelia burgdorferi* sensu lato (s.l.), and Anaplasmatacea, although other bacteria, protozoa and virus have also been described. For some of these but also for other tick-borne agents, a medical role was suggested by the detection of hosts harboring infected feeding ticks or presenting specific antibodies that could potentially be linked to agents' exposure (Amaro *et al.* 2017). Moreover, autochthonous human clinical cases were already reported for the tick-borne *Babesia divergens*, *Borrelia burgdorferi* s.l., *Rickettsia conorii*, *R. sibirica* mongolotimonae and *R. slovaca* (Bacellar *et al.* 2003; Centeno-Lima *et al.* 2003; Da Franca *et al.* 2005; De Sousa *et al.* 2003, 2006, 2013; Lopes de Carvalho *et al.* 2006). Regarding companion animal, infections by *Anaplasma platys*, *B. burgdorferi* s.l., *Ehrlichia canis*, *R. conorii*, *Cytauxzoon* sp., *Hepatozoon canis*, *Babesia canis* and *B. vulpes* were detected in symptomatic cases (Alexandre *et al.* 2008; Alexandre *et al.* 2011; Cardoso *et al.* 2008; Santos *et al.* 2009; Simões *et al.* 2011; Maia *et al.* 2015; Alho *et al.* 2016b, 2016f). In livestock industry, the circulation of several tick-borne agents has also been reported, including *Babesia bovis*, *B. bigemina*, *B. divergens*, *Theileria annulata* and *T. buffeli* (Brígido *et al.* 2004; Gomes *et al.* 2013) and LPDP team co-authored detection guidelines for these agents (Lempereur *et al.* 2017; Portillo *et al.* 2017; Silaghi *et al.* 2017).

As for other arthropod vectors, proper species identification is extremely important in the study of ticks. The taxonomic identification has been traditionally supported by



**Table 2.** List of portuguese ticks species and the agents that have been found in questing ticks collected across the country

Tick	Agents' Order/Species				Piroplasmida <sup>e</sup>
Genus/Species	Bunyavirales <sup>a</sup>	Rickettsiales <sup>b</sup>	Legionellales <sup>c</sup>	Spirochaetales <sup>d</sup>	
<b><i>Dermacentor</i></b>					
<i>D. marginatus</i>	<i>Phlebovirus</i> sp.	<i>Anaplasma marginale</i> ; <i>Rickettsia slovacica</i> ; <i>Rickettsia raoulti</i>	<i>Coxiella burnetii</i> ; <i>Francisella tularensis</i> subsp. <i>Holarctica</i> (Fth)	<i>Borrelia lusitaniae</i>	
<i>D. reitculatus</i>		<i>R. slovacica</i>	<i>F. tularensis</i> (Fth); <i>Francisella</i> -like endosymbiont		
<b><i>Haemaphysalis</i></b>					
<i>H. hispanica</i>					
<i>H. inermis</i>					
<i>H. punctata</i>				Relapsing Fever-like <i>Borrelia</i> sp.	
<b><i>Hyalomma</i></b>					
<i>H. lusitanicum</i>		<i>Anaplasma platys</i> ; <i>Candidatus</i> <i>Mitichloria mitochondrii</i>	<i>C. burnetii</i>		
<i>H. marginatum</i>				<i>B. lusitaniae</i>	
<b><i>Ixodes</i></b>					
<i>I. acuminatus</i>					
<i>I. arboricola</i>					

(continued)

Table 2. (continued)

Tick	Agents' Order/Species			Spirochaetales <sup>d</sup>	Piroplasmida <sup>e</sup>
	Bunyavirales <sup>a</sup>	Rickettsiales <sup>b</sup>	Legionellales <sup>c</sup>		
<i>I. canisuga</i>					
<i>I. frontalis</i>			<i>C. burnetii</i>		
<i>I. hexagonus</i>			<i>Francisella</i> -like endosymbiont		
<i>I. inopinatus</i>					
<i>I. ricinus</i>		<i>Anaplasma phagocytophilum</i> ; <i>A. marginale</i> ; <i>Rickettsia helvetica</i> ; <i>Rickettsia monacensis</i>	<i>C. burnetii</i> ; <i>F. tularensis</i> (Fth)	<i>Borrelia afzelii</i> ; <i>Borrelia garinii</i> ; <i>Borrelia burgdorferi</i> s.s.; <i>B. lusitanae</i> ; <i>Borrelia miyamotoi</i> ; <i>Borrelia valaisiana</i>	
<i>I. simplex</i>					
<i>I. ventraloi</i>		<i>A. phagocytophilum</i> ; <i>A. marginale</i> ; <i>Candidatus Neohrlichia mikurensis</i> -like; <i>R. helvetica</i>	<i>C. burnetii</i>		<i>Theileria annulata</i>
<i>I. vespertilionis</i>					
<b><i>Rhipicephalus</i></b>					
<i>R. annulatus</i>					
<i>R. bursa</i>		<i>A. marginale</i>			
<i>R. pusillus</i>		<i>Rickettsia sibirica mongolitimonae</i>			

(continued)

**Table 2.** (continued)

Tick	Agents' Order/Species				
Genus/Species	Bunyavirales <sup>a</sup>	Rickettsiales <sup>b</sup>	Legionellales <sup>c</sup>	Spirochaetales <sup>d</sup>	Piroplasmida <sup>e</sup>
<i>R. sanguineus</i> s.l.	<i>Phlebovirus</i> sp.	<i>A. marginale</i> ; <i>Rickettsia conorii</i> ; <i>Rickettsia massiliae</i>	<i>F. tularensis</i> (Fth)	Relapsing Fever-like <i>Borrelia</i> sp.	

<sup>a</sup>Pereira *et al.* 2017;

<sup>b</sup>Antunes *et al.* 2016; Bacellar *et al.* 1995a, b; Ferrolho *et al.* 2016; Milhano *et al.* 2010; REVIVE 2014, 2017; Santos *et al.* 2004; Santos-Silva *et al.* 2017; Santos *et al.* 2018;

<sup>c</sup>Lopes de Carvalho *et al.* 2016; Santos-Silva *et al.* 2017; Santos *et al.* 2018;

<sup>d</sup>Núncio *et al.* 1993; Matuschka *et al.* 1998; De Michelis *et al.* 2000; Baptista *et al.* 2004; Nunes *et al.* 2015, 2016;

<sup>e</sup>Antunes *et al.* 2016

the observation of particular morphological features that are regarded as diagnostic characters and constitute the basis of ticks' dichotomous identification keys (examples of these features are the shape and size of the base of the *capituli*, the presence/absence and shape of spurs, *auriculae* and *cornua*). The classic classification should be undertaken through careful morphological features identification before any further study. Recently, in a comparative blind test study performed by several European tick experts, it was possible to ascertain that if the researcher was not familiar with a given species, it might be misidentified (Estrada-Peña *et al.* 2017). This elucidates the need to promote adequate training for experts in tick identification. The molecular analysis done upon stable identification of ticks complement this process and can be based in the study of conserved and moderately conserved genes, as the mitochondrial 12S and 16S rRNA or others such as the second internal transcribed rDNA spacer (ITS2) (Estrada-Peña *et al.* 2017; Santos-Silva *et al.* 2017; Santos *et al.* 2018; Sanches *et al.* 2018). This integrated approach of identification is highlighting a genetic heterogeneity of some tick populations that in some instances is questioning species definition, as in the case of *Rhipicephalus sanguineus*/*R. turanicus*, *I. ricinus*/*I. inopinatus* and possibly *I. frontalis* (Santos-Silva *et al.* 2011; Estrada-Peña *et al.* 2014; Santos *et al.* 2018). Differences in vector competence are also discussed for distinct genotypic lineages of a given species, like the case of *R. sanguineus* concerning *E. canis* transmission (Sanches *et al.* 2018). Thus, particular attention is devoted to mega vector *I. ricinus* and to the characterization of its population's genetics and the differences in vector potential of distinct genetic lineages. In this regard, the Portuguese Foundation for Science and Technology has recently financed a project entitled "The *Ixodes ricinus* group of ticks in the western Mediterranean region and North Africa: new insights into their population genetics and microbiome fauna – TickGenoMi (ref PTDC/SAU-PAR/28947/2017)". This project combines the taxonomic issues regarding interspecific diversity and potential gene flow among populations of *I. ricinus*/*I. inopinatus* with the study of their microbial community.

**An Update on Cattle Piroplasmiasis.** Belonging to phylum Apicomplexa, order Piroplasmida, *Babesia* and *Theileria* parasites are responsible for important diseases with great economic and social impact (Castro 1997; Gubbels 1999). Protozoan parasites of genus *Babesia* are responsible for bovine babesiosis, with *B. bovis* and *B. bigemina* being the most common and studied species that affect cattle worldwide, with *B. bovis* causing more severe disease than *B. bigemina*. *B. divergens* also infects cattle but, unlike the others, it has a zoonotic potential and is one of the species responsible for human babesiosis. Bovine babesiosis has had a huge economic impact, causing losses in beef production due to animal disease and death. There are also other associated costs such as the high cost of tick control, disease detection, prevention and treatment (Yusuf 2017). *Theileria* species are responsible for Bovine theileriosis which is caused by several *Theileria* species such as *T. parva*, responsible for East Coast fever in Africa and *T. annulata*, that causes Tropical Theileriosis, which are the most pathogenic and economically important species. Tropical Theileriosis is widely distributed in the Mediterranean basin, including southern Europe and northern Africa, and also many countries from the Middle East and Asia, such as India and China. Animals that survive the acute disease usually become chronic carriers and play an important role as a reservoir for the maintenance of both parasites' life cycle (Garcia-Sanmartin *et al.* 2006).

Different studies confirmed the occurrence of *Babesia* and *Theileria* parasites in Portugal (Brígido *et al.* 2004; Silva *et al.* 2010). However, for a broader picture of piroplasms' prevalence and distribution in Portugal, an epidemiological survey was conducted to assess the *Theileria* and *Babesia* species infecting bovines in mainland Portugal (Gomes *et al.* 2013). The global prevalence of piroplasm-infected animals was 36.8%, although significant differences were found between the north and south of the country. A higher prevalence was found in the southern regions, with 42.4% infected animals in Lisbon and 51.6% in Alentejo. These differences are certainly related to the distinct vegetation and climatic characteristics of those regions, influencing vector tick distribution and abundance across the country and, as a result, the incidence of piroplasmosis. Different livestock management systems amongst regions can also influence the exposure of animals to vector ticks. *Theileria annulata* was the most frequently found species, with a national prevalence of 21.3%, with a higher regional value in Lisbon (33.5%) and Alentejo (29.2%). The majority of reported clinical cases occur in young calves or imported animals, therefore, a state of endemic stability is presumed to occur. In the northern part of the country, where infected animals and vector ticks are also known to co-exist, the disease is less common and tick challenge is presumed to be relatively lower. *Theileria orientalis* was the second most prevalent species found, with 10.1% infected animals, but there are no reports of clinical cases in Portugal due to this species, supporting the idea that it is non-pathogenic to bovines. Since several *T. orientalis* genotypes are reported to be pathogenic to cattle, this parasite's population should be studied to assess its role in cattle's health (Kamau 2011). A low prevalence was detected for *Babesia* infections (7.9%), with *B. bigemina* being the most frequently found species. *B. bovis*, which is usually considered the most pathogenic bovine *Babesia* was absent. *B. divergens* was detected in one animal from Alentejo region (Gomes *et al.* 2013).

Given the potential threat of cattle piroplasmosis to the livestock industry, an enhanced awareness of the epidemiological traits of all of different species is essential for assessing exposure risks and a better planning of prophylactic and control measures. Therefore, the improvement of diagnostic techniques and development of new methods are fundamental to the control of all tick-borne pathogens. Traditionally, detection of *Theileria* and *Babesia* pathogens in infected animals is accomplished by microscopic examination of stained blood smears, which have low sensitivity for the assessment of carrier animals, in which low numbers of erythrocytes remain infected (Altay *et al.* 2008). Although serological methods can be employed to diagnose subclinical infections, cross-reactions are common and current infections and previous exposures are generally not distinguished, moreover, antibodies tend to disappear in long-term carriers (Altay *et al.* 2008; García-Sanmartín *et al.* 2006). Different molecular diagnostic-based assays have also been developed, some of which have proven to be effective in detecting piroplasm infections in carrier animals. The reverse line blotting (RLB) assay based on the amplification of the hyper-variable V4 region of the 18S rDNA gene of *Theileria* and *Babesia* parasites and reverse hybridization of the products with species-specific oligonucleotide probes is currently considered to be the most sensitive test for detecting these parasites (Gubbels *et al.* 1999; Georges *et al.* 2001; Bilgic *et al.* 2010). Nevertheless, since RLB is a relatively cumbersome assay, it is not entirely suitable for use in the routine diagnosis

of piroplasm infections. Therefore, different PCR-based assays have also been described for detecting all the diversity of *Theileria* and *Babesia* parasites (d'Oliveira *et al.* 1995; Criado-Fornelio *et al.* 2009). Focusing in the most prevalent species infecting bovines in Portugal, a study was developed to review the *in silico* efficiency of previously described primers, to design new primers for amplifying the *T. annulata* species-specific Tams1-encoding gene and to develop and validate an efficient real-time PCR assay for detecting this protozoan in bovine blood samples (Santos *et al.* 2013). This real-time PCR assay proved to be 100% specific taking the RLB as gold standard.

The studies clearly demonstrated that the reassessment of all primers and probes used in molecular diagnostic assays for targeting pathogen genes should be performed periodically, as novel relevant nucleotide sequences become available from public databases. This was demonstrated by the design of an updated primer set targeting more conserved regions of the Tams1 gene of *T. annulata* (Santos *et al.* 2013). Besides these studies, other methods are being developed such as the Loop mediated isothermal amplification molecular assays. For the specific detection of *T. annulata* in bovine blood samples by targeting the Tams1 gene, a LAMP assay was developed, and a field study was performed where the Tams1-targeted assay was validated using a large set of blood samples collected from cattle in a theileriosis endemic area at southern Portugal and the real-time PCR was used to estimate parasitaemia in naturally infected cattle (Gomes *et al.* 2017). The overall sensitivity of the LAMP assay was estimated as 90%, with negative and positive predictive values of 87% and 100%, respectively, in agreement with the real-time PCR results. The LAMP assay was able to detect *T. annulata* DNA in blood samples with a very low parasitaemia, with a value of 0.00026% considered as the detection limit of this assay. None of the animals in this study showed clinical signs of disease and were considered healthy carriers, which in natural infection usually result in parasitaemias of approximately 0.1 to 0.01% in carrier animals (Aktas *et al.* 2006). When analysing the real-time PCR results and the correlated parasitaemia, the values correspond to a parasitaemia with a mean value of 0.009%, which are difficult to detect by microscopy of blood smears. These studies demonstrate the usefulness of diagnostic methods in the evaluation of different aspects of an important tick-borne disease of cattle.

Theileriosis importance is becoming increasingly recognised in Portugal and, since epidemiological studies have shown a rise in prevalence in the south (Branco *et al.* 2010; Silva *et al.* 2010; Gomes *et al.* 2013), a study was designed to assert *T. annulata* population genetics' with the goal of investigating the genetic diversity of this parasite in Portugal and to perform a comparative analysis with the data available from Tunisia and Turkey (Gomes *et al.* 2016). This parasite has considerable levels of genetic diversity within its population, as shown by different studies using a variety of serological and molecular methods (Shiels *et al.* 1986; Ben-Miled *et al.* 1994; Weir *et al.* 2007). For the Portuguese cattle population studies, samples were collected from twelve farms at four distinct regions. The panel of micro- and mini-satellites was previously designed to investigate the structure of parasite population in relation to the different geographical regions, but also to evaluate the multiplicity of infection of *T. annulata* genotypes in the cattle hosts (Weir *et al.* 2011). The results show that the parasite population is highly diverse, similar to what happens in other endemic countries and there is some genetic differentiation between geographically separated populations of *T. annulata*. A

moderate level of differentiation was detected between Portugal and Tunisia and between Portugal and Turkey. While Portugal does not experience the same problem of tropical theileriosis in comparison to other Mediterranean countries, it is clear that the extent of genetic diversity of *T. annulata* is similar to countries where the disease represents one of the principal constraints to cattle production. Population genetics' information may provide important baseline data upon which suitable Theileriosis control policies may be developed in the near future.

***Dirofilaria immitis* vs. *Angiostrongylus vasorum* in Portugal: Current Epidemiological Situation and the Status of Co-infections with Important Vector-Borne Pathogens.**

*D. immitis* and *A. vasorum* are severe and life-threatening cardiopulmonary nematodes of pets, which have increasingly been reported throughout Europe (Traversa *et al.* 2010; Alho *et al.* 2018). Several factors have been suggested for this expansion, namely incremented global transports, demographic and political changes, urbanization, increasing density of vulpine reservoir host populations, climate changes and the availability of better diagnostic tools (Otranto *et al.* 2013). *D. immitis* (Leidy 1856) (Spirurida, Onchocercidae) is a potentially zoonotic vector-borne pathogen, transmitted by multiple species of mosquitoes (Culicidae), and it is the causative agent of canine cardiopulmonary dirofilariosis/heartworm disease. Differently, *A. vasorum* (Baillet 1866) (Metastrongyloidea, Angiostrongylidae) is a non-zoonotic nematode, transmitted by gastropod mollusks and it is the causative agent of canine angiostrongylosis (Guilhon and Cens 1973). Information about the prevalence and distribution of cardiopulmonary parasites is essential for the prevention of animal and potentially associated human diseases, particularly considering their growing incidence and clinical severity. However, in Portugal, accurate data on both illnesses was scarce and limited to a few studies and case reports. Therefore, a multidisciplinary study was designed to characterize and assess the national situation regarding dirofilariosis and angiostrongylosis in canine and red fox populations from Portugal. This study allowed to confirm the occurrence of both diseases in canids at northern, central and southern regions of Portugal, with an overall prevalence of 11.9% dogs and 8.5% foxes positive to *D. immitis*, with higher prevalences registered in Center South and Algarve (Alho *et al.* 2014a, 2016a, 2016b, 2018). *A. vasorum* was throughout the country but with a lower prevalence, 0.7% positive dogs (Alho *et al.* 2016c) and 12.7% positive foxes (Alho *et al.* 2017a). This study showed that although exposure may differ depending on the region of Portugal, the likelihood of both infections is a possibility nationwide. Additionally, the transmission risk period for *Dirofilaria* spp. was estimated in Portugal using a degree-day model, based on the temperatures registered at five meteorological stations in Portugal over a decade. Overall, Madeira Island was found to be the area registering the highest number of days with suitable conditions for *D. immitis* transmission, followed by Faro, Lisbon, Azores and Oporto. As expected, higher average temperatures were observed predominantly in Madeira and in the southern parts of the country. Although risk was found to be markedly seasonal (predominantly in the summer), this study evidenced that in Portugal the transmission period is starting earlier and lasting far beyond the warmest months of the year (Alho *et al.* 2014b). This is in line with the “seasonality paradigm” of vector-borne diseases that shows their occurrence is no longer a seasonal phenomenon (Otranto *et al.* 2013). In parallel and showing once again the southern trend of this disease in

Portugal and the risk for unusual hosts, a high prevalence of *D. immitis* was found in pinnipeds kept at an oceanographic park in the Algarve region. Overall, common seals (*Phoca vitulina*), California sea lions (*Zalophus californianus*) and South African fur seals (*Arctocephalus pusillus pusillus*) were positive for *D. immitis* by real-time PCR, representing an overall prevalence of 43.8% (Alho *et al.* 2017b). This high prevalence detected in a confined area located in a popular summer destination represents a risk interface for zoonotic pathogen transmission, and an example of how a One Health approach is vital to improve early diagnosis and control of zoonotic pathogens in humans and wildlife. The treatment goals of dirofilariosis are to improve the animal's clinical condition and to eliminate all life stages of the parasite with minimal post treatment side effects. This can be achieved through mechanical, surgical or chemotherapeutic approaches. Manual extraction is the preferred method due to its diminished invasiveness, reduced damage to the vascular endothelium and shortened anesthesia duration (Atkins 2010). However, it is an expensive technique that can be highly traumatic when extraction brushes are used. To overcome this issue, a new minimally invasive surgical technique was developed to extract *D. immitis* adult worms from the hearts of dogs through transjugular catheterization with a non-traumatic snare (Alho *et al.* 2016d). A 0.014-inch coronary wire (BMW, Abbott Vascular) was adapted, allowing the successful transvenous extraction of *D. immitis* adult specimens from the pulmonary artery and right ventricle of a severely infected dog. Further surgical interventions need to be performed to improve the efficiency of this technique. Nevertheless, we defend that the possible cost reductions and diminished traumatic damage induced by this snare, will allow heartworm extraction to be more affordable and consequently widespread, thereby promoting the treatment of a larger number of animals, enhancing a specific chemotherapy with higher safety. Furthermore, a questionnaire conducted on Portuguese pet owners at a veterinary hospital in Portugal showed that the majority deworm their dogs at irregular and consequently ineffective intervals. Only 11.8% of the dogs were under the recommended endoparasitic treatment (i.e. quarterly, at least) and only 28.4% were continuously protected throughout the year from vector-borne agents. Moreover, 60% of the owners who kept their dogs outdoors the entire day did not perform adequate ectoparasitic prevention on their animals. Besides, the level of public knowledge in Portugal about parasites and parasitic diseases is still low, i.e. 88% had never heard of "dirofilariosis/heartworm disease" and 85% had never heard of "zoonosis" (Matos *et al.* 2015).

Emerging VBDs and co-infections with other important vector-borne pathogens are being increasingly reported worldwide. That is the case of *Onchocerca lupi* (Rodonaja 1967) (Spirurida, Onchocercidae), a helminth that causes nodular lesions associated with acute or chronic ocular disease in dogs and cats (Grácio *et al.* 2015). Since its first description in 1991, this zoonotic filarioid has been found with increasing frequency in canine and feline species across Europe and the United States. Recently we reported an ectopic location of canine onchocercosis featured by an atypical clinical presentation of dyspnea. An exploratory laryngoscopy revealed a laryngeal nodule causing a severe reduction of the trachea, along with several filiform worms, later morphologically and molecularly identified as *O. lupi* (Alho *et al.* 2016e). This was the first report of an aberrant migration of *O. lupi* in a dog, alerting the veterinary medical community to the occurrence of erratic locations of virulent zoonotic nematodes. Other life-threatening vector-borne parasite is



*Cytauxzoon* sp., an haemoprotozoan pathogen of the genus *Cytauxzoon* (Theileriidae) that is transmitted by ticks (Lloret *et al.* 2015). Although in literature information is scant, a case of *Cytauxzoon* sp. infection was recently diagnosed for the first time in Portugal in a domestic felid (Alho *et al.* 2016f). The cat was presented with an history of acute lethargy, anorexia, pyrexia and severe anaemia. A molecular screening for the detection of causative agents of infectious anaemia showed a positive result for Piroplasmorida, with DNA sequencing of the 18S rRNA gene revealing 99.9% identity with *Cytauxzoon manul*. Besides being emerging, VBDs agents infect concomitantly the same host and in a recent study, two thirds of the surveyed dogs showed at least one agent and the number of VBDs ranged from 3–4 in the islands and 5–7 in mainland Portugal (Alho *et al.* 2016b). The transmission behavior of these diseases is highly dependent on their vectors and their integrated study should be performed continuously.

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## References

- Aktas, M., Altay, K., Dumanli, N.: A molecular survey of bovine *Theileria* parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey. *Vet. Parasitol.* **138**(3–4), 179–85 (2006)
- Alexandre, N., Santos, A.S., Bacellar, F., *et al.*: Detection of *Rickettsia conorii* strains in Portuguese dogs (*Canis familiaris*). *Ticks Tick Borne Dis.* **2**(2), 119–122 (2011)
- Alexandre, N., Santos, A.S., Nuncio, M.S., *et al.*: Detection of *Ehrlichia canis* by polymerase chain reaction in dogs from Portugal. *Vet J.* **181**(3), 343–344 (2008)
- Alexandre-Pires, G., Ramilo, D., Diaz, S., *et al.*: Investigating morphological structures of *Culicoides* from Obsoletus complex by using Scanning Electron Microscopy and Composed Optical Microscopy. In: Méndez-Vilas, A., Álvarez, J.D. (eds.) *Microscopy: Science, Technology, Applications and Education*, vol. 2, pp. 792–802. Formatex Research Center (2010)
- Alho, A.M.: *Dirofilaria immitis* and *Angiostrongylus vasorum*: epidemiology and impact of major heartworms in carnivores in Portugal. Ph.D. thesis. Faculdade de Medicina Veterinária, Universidade de Lisboa, 25 July 2017, 227 pp., Lisbon (2017a). <https://www.repository.utl.pt/handle/10400.5/13975>
- Alho, A.M., Cortes, H., Lopes, A.P., *et al.*: Detection of *Dirofilaria immitis* antigen in red foxes (*Vulpes vulpes*) from Portugal. *Parasites Vectors* **10**(1), A16 (2016a)

- Alho, A.M., Cruz, L., Coelho, A., et al.: Aberrant laryngeal location of *Onchocerca lupi* in a dog. *Parasitol. Int.* **65**(3), 218–220 (2016e)
- Alho, A.M., Fiarresga, A., Landum, M., et al.: A homemade snare: an alternative method for mechanical removal of *Dirofilaria immitis* in dogs. *Vet. Med. Int.* Article no. 5780408 (2016d)
- Alho, A.M., Landum, M., Ferreira, C., et al.: Prevalence and seasonal variations of canine dirofilariosis in Portugal. *Vet. Parasitol.* **206**, 99–105 (2014a)
- Alho, A.M., Marcelino, I., Colella, V., et al.: *Dirofilaria immitis* in pinnipeds and new host record. *Parasites Vectors* **10**, 142 (2017b)
- Alho, A.M., Meireles, J., Schnyder, M., et al.: *Dirofilaria immitis* and *Angiostrongylus vasorum*: the current situation of two major canine heartworms in Portugal. *Vet. Parasitol.* **252**, 120–126 (2018)
- Alho, A.M., Nunes, T., Rinaldi, L., et al.: Transmission risk of Dirofilariosis in Portugal. *Parasites Vectors* **7**(1), O16 (2014b)
- Alho, A.M., Pita, J., Amaro, A., et al.: Seroprevalence of vector-borne pathogens and molecular detection of *Borrelia afzelii* in military dogs from Portugal. *Parasites Vectors* **9**(1), 225 (2016b)
- Alho, A.M., Schnyder, M., Schaper, R., et al.: Seroprevalence of circulating *Angiostrongylus vasorum* antigen and parasite-specific antibodies in dogs from Portugal. *Parasitol. Res.* **115**, 2567–2572 (2016c)
- Alho, A.M., Silva, J., Fonseca, M.J., et al.: First report of *Cytauxzoon* sp. infection in a domestic cat from Portugal. *Parasites Vectors* **9**(1), 220 (2016f)
- Altay, K., Aydin, M.F., Dumanli, N., Aktas, M.: Molecular detection of *Theileria* and *Babesia* infections in cattle. *Vet. Parasitol.* **158**(4), 295–301 (2008)
- Amaro, A., Patricio, C., Nunes, M., Carreira, T., Vieira, M.L.: Lyme borreliosis in asymptomatic animals: serological and molecular detection of *Borrelia burgdorferi* sensu lato. In: Final Conference on Neglected Vectors and Vector-Borne Diseases (Eurnegvec) with Management Committee and Working Group Meetings of the COST Action TD1303, Abstract Book, Chania, 11–13 September 2017, pp. 3 (2017)
- Antunes, S., Ferrolho, J., Domingues, N., et al.: *Anaplasma marginale* and *Theileria annulata* in questing ticks from Portugal. *Exp. Appl. Acarol.* **70**(1), 79–88 (2016)
- Ash, J.S.: A study of the Mallophaga of birds with particular reference to their ecology. *Ibis (Lond 1859)* **102**, 93–110 (1960). <https://doi.org/10.1111/j.1474-919x.1960.tb05095.x>
- Atkins, C.: Canine heartworm disease. In: Ettinger, S., Feldman, E. (eds.) *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*, 2nd edn., pp. 1353–1381. Saunders Elsevier, St. Louis (2010)
- Bacellar, F., Sousa, R., Santos, A., et al.: Boutonneuse fever in Portugal: 1995–2000. Data of a state laboratory. *Eur. J. Epidemiol.* **18**(3), 275–277 (2003)
- Bacellar, F., Nuncio, M.S., Alves, M.J., Filipe, A.R.: *Rickettsia slovaca*: un agente del grupo de las fiebres exantemáticas en Portugal. *Enfermedades Infecciosas y Microbiología Clínica* **13**, 218–223 (1995a)
- Bacellar, F., Regnery, R.L., Nuncio, M.S., Filipe, A.R.: Genotypic evaluation of rickettsial isolates recovered from various species of ticks in Portugal. *Epidemiol. Infect.* **114**, 169–178 (1995b)
- Balenghien, T., Pagès, N., Goffredo, M., et al.: The emergence of Schmallenberg virus across *Culicoides* communities and ecosystems in Europe. *Prev. Vet. Med.* **116**, 360–369 (2014)
- Baptista, S., Quaresma, A., Aires, T., et al.: Lyme borreliosis spirochetes in questing ticks from mainland Portugal. *Int. J. Med. Microbiol.* **293**, 109–116 (2004)
- Barros, S.C., Ramos, F., Luís, T.M., et al.: Molecular epidemiology of bluetongue virus in Portugal during 2004–2006 outbreak. *Vet. Microbiol.* **124**, 25–34 (2007)
- Bartlett, C.M.: Lice (Amblycera and Ischnocera) as Vectors of Eulimdana spp. (Nematoda: Filarioidea) in Charadriiform Birds and the Necessity of Short Reproductive Periods in Adult Worms. *J. Parasitol.* **79**, 85 (1993). <https://doi.org/10.2307/3283282>

- Bartlett, C.M., Anderson, R.C.: *Pelecitus fulicaeatrae* (Nematoda: Filarioidea) of coots (Gruiformes) and grebes (Podicipediformes): skin-inhabiting microfilariae and development in Mallophaga. *Can. J. Zool.* **65**, 2803–2812 (1987). <https://doi.org/10.1139/z87-423>
- Ben-Miled, L., Dellagi, K., Bernardi, G., et al.: Genomic and phenotypic diversity of Tunisian *Theileria annulata* isolates. *Parasitology* **108**(Pt 1), 51–60 (1994)
- Bilgic, H.B., Karagenç, T., Shiels, B., et al.: Evaluation of cytochrome b as a sensitive target for PCR based detection of *T. annulata* carrier animals. *Vet. Parasitol.* **174**(3–4), 341–347 (2010)
- Bonser, R.H.: Mites on birds. *Trends Ecol. Evol.* **16**, 18–19 (2001). [https://doi.org/10.1016/S0169-5347\(00\)02012-7](https://doi.org/10.1016/S0169-5347(00)02012-7)
- Booth, D.T., Clayton, D.H., Block, B.A.: Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proc. R. Soc. B Biol. Sci.* **253**, 125–129 (1993). <https://doi.org/10.1098/rspb.1993.0091>
- Branco, S., Orvalho, J., Leitão, A., et al.: Fatal cases of *Theileria annulata* infection in calves in Portugal associated with neoplastic-like lymphoid cell proliferation. *J. Vet. Sci.* **11**(1), 27–34 (2010)
- Brígido, C., da Fonseca, I.P., Parreira, R., et al.: Molecular and phylogenetic characterization of *Theileria* spp. parasites in autochthonous bovines (Mirandesa breed) in Portugal. *Vet. Parasitol.* **123**(1–2), 17–23 (2004)
- Cardoso, L., Costa, A., Tuna, J., et al.: *Babesia canis canis* and *Babesia canis vogeli* infections in dogs from northern Portugal. *Vet. Parasitol.* **156**(3–4), 199–204 (2008)
- Centeno-Lima, S., do Rosário, V., Parreira, R., et al.: A fatal case of human babesiosis in Portugal: molecular and phylogenetic analysis. *Trop. Med. Int. Health* **8**(8), 760–764 (2003)
- Clayton, D.H., Adams, R.J., Bush, S.E.: Phthiraptera, the chewing lice. In: Thomas, N.J., Hunter, D.B., Atkinson, C.T. (eds.) *Parasitic Diseases of Wild Birds*, 1st edn., pp 515–526. Wiley-Blackwell, Iowa (2008)
- Clayton, D.H., Bush, S.E., Johnson, K.J.: Ecology of congruence: past meets present. *Syst. Biol.* **53**, 165–173 (2004). <https://doi.org/10.1080/10635150490265102>
- Criado-Fornelio, A., Buling, A., Asenzo, G., et al.: Development of fluorogenic probe-based PCR assays for the detection and quantification of bovine piroplasmids. *Vet. Parasitol.* **162**(3–4), 200–206 (2009)
- Da Franca, I., Santos, L., Mesquita, T., et al.: Lyme borreliosis in Portugal caused by *Borrelia lusitanae*? Clinical report on the first patient with a positive skin isolate. *Wien. Klin. Wochenschr.* **117**(11–12), 429–432 (2005)
- de Castro, J.J.: Sustainable tick and tickborne disease control in livestock improvement in developing countries. *Vet. Parasitol.* **71**(2–3), 77–97 (1997)
- De Michelis, S., Sewell, H.S., Collares-Pereira, M., et al.: Genetic diversity of *Borrelia burgdorferi* sensu lato in ticks from mainland Portugal. *J. Clin. Microbiol.* **38**(6), 2128–2133 (2000)
- De Sousa, R., Barata, C., Vitorino, L., et al.: *Rickettsia sibirica* isolation from a patient and detection in ticks, Portugal. *Emerg. Infect. Dis.* **12**(7), 1103–1108 (2006)
- De Sousa, R., Nóbrega, S.D., Bacellar, F., Torgal, J.: Mediterranean spotted fever in Portugal: risk factors for fatal outcome in 105 hospitalized patients. *Ann. N. Y. Acad. Sci.* **990**, 285–294 (2003)
- De Sousa, R., Pereira, B.I., Nazareth, C., et al.: *Rickettsia slovaca* infection in humans, Portugal. *Emerg. Infect. Dis.* **19**(10), 1627–1629 (2013). <https://doi.org/10.3201/eid1910.130376>
- Derylo, A.: Mallophaga as a reservoir of *Pasteurella multocida*. *Acta Parasitologica Polonica* **17**, 301–313 (1970)
- d'Oliveira, C., van der Weide, M., Habela, M.A., Jacquiet, P., Jongejan, F.: Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. *J. Clin. Microbiol.* **33**(10), 2665–2669 (1995)

- Estrada-Peña, A., D'Amico, G., Palomar, A.M., et al.: A comparative test of ixodid tick identification by a network of European researchers. *Ticks Tick Borne Dis.* **8**(4), 540–546 (2017). <https://doi.org/10.1016/j.tbd.2017.03.001>
- Estrada-Peña, A., Nava, S., Petney, T.: Description of all the stages of *Ixodes inopinatus* n. sp. (Acari: Ixodidae). *Ticks Tick Borne Dis.* **5**(6), 734–743 (2014). <https://doi.org/10.1016/j.tbd.2014.05.003>
- Ferrolho, J., Antunes, S., Santos, A.S., et al.: Detection and phylogenetic characterization of *Theileria* spp. and *Anaplasma marginale* in *Rhipicephalus bursa* in Portugal. *Ticks Tick Borne Dis.* **7**(3), 443–448 (2016). <https://doi.org/10.1016/j.tbd.2016.01.004>
- García-Sanmartín, J., Nagore, D., García-Pérez, A.L., et al.: Molecular diagnosis of *Theileria* and *Babesia* species infecting cattle in Northern Spain using reverse line blot macroarrays. *BMC Vet. Res.* **2**, 16 (2006)
- Georges, K., Loria, G.R., Riili, S., et al.: Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. *Vet. Parasitol.* **99**(4), 273–286 (2001)
- Gomes, J., Salgueiro, P., Inácio, J., et al.: Population diversity of *Theileria annulata* in Portugal. *Infect. Genet. Evol.* **42**, 14–19 (2016)
- Gomes, J., Santos, M., Amaro, A., et al.: A field evaluation of an isothermal DNA amplification assay for the detection of *Theileria annulata* infection in cattle. *Mol. Cell. Probes* **31**, 61–64 (2017)
- Gomes, J., Soares, R., Santos, M., et al.: Detection of *Theileria* and *Babesia* infections amongst asymptomatic cattle in Portugal. *Ticks Tick Borne Dis.* **4**(1–2), 148–151 (2013)
- Grácio, A.J., Richter, J., Komnenou, A.T., Grácio, M.A.: Onchocerciasis caused by *Onchocerca lupi*: an emerging zoonotic infection. Systematic review. *Parasitol. Res.* **114**, 2401–2413 (2015)
- Gubbels, J.M., de Vos, A.P., van der Weide, M., et al.: Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. *J. Clin. Microbiol.* **37**(6), 1782–1789 (1999)
- Guilhon, J., Cens, B.: *Angiostrongylus vasorum* (Baillet: 1866). Étude biologique et morphologique. *Annales de Parasitologie Humaine et Comparee* **48**, 567–596 (1973)
- Hillgarth, N.: Ectoparasite transfer during mating in ring-necked Pheasants *Phasianus colchicus*. *J. Avian Biol.* **27**, 260 (1996). <https://doi.org/10.2307/3677232>
- Howitt, B.F., Dodge, H.R., Bishop, L.K., Gorrie, R.H.: Virus of Eastern Equine Encephalomyelitis Isolated from Chicken Mites (*Dermanyssus gallinae*) and Chicken Lice (*Eomenacanthus stramineus*). *Exp. Biol. Med.* **68**, 622–625 (1948). <https://doi.org/10.3181/00379727-68-16577>
- Jacquet, S., Garros, C., Lombaert, E., et al.: Colonization of the mediterranean basin by the vector biting midge *Culicoides imicola*: an old story. *Mol. Ecol.* **24**, 5707–5725 (2015)
- Jacquet, S., Huber, K., Pagès, N., et al.: Range expansion of the Bluetongue vector, *Culicoides imicola*, in continental France likely due to rare wind-transport events. *Sci. Rep.* **6**, 27247 (2016)
- Johnson, K.P., Clayton, D.H.: The biology, ecology, and evolution of chewing lice. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., et al. (eds.) *The Chewing Lice: World Checklist and Biological Overview*, pp. 449–476. Illinois Natural History Survey Special Publication, Champaign (2003)
- Kamau, J., de Vos, A.J., Playford, M., et al.: Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. *Parasites Vectors* **4**, 22 (2011)
- Keirans, J.E.: A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). *J. Med. Entomol.* **12**, 71–76 (1975). <https://doi.org/10.1093/jmedent/12.1.71>
- Kose, M., Mänd, R., Møller, A.: Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. *Anim. Behav.* **58**, 1201–1205 (1999). <https://doi.org/10.1006/anbe.1999.1249>

- Linley, J.R., Hoch, A.L., Pinheiro, F.P.: Biting midges (Diptera: Ceratopogonidae) and human health. *J. Med. Entomol.* **20**, 347–364 (1983)
- Lempereur, L., Beck, R., Fonseca, I., et al.: Guidelines for the detection of *Babesia* and *Theileria* parasites. *Vector-Borne Zoonotic Dis.* **17**(1), 51–65 (2017). Guidelines Issue, Edit. Stephen Higgs
- Literák, I., Sychra, O., Resendes, R., Rodrigues, P.: Chewing lice in Azorean blackcaps (*Sylvia atricapilla*): a contribution to Parasite Island syndromes. *J. Parasitol.* **101**, 252–254 (2015). <https://doi.org/10.1645/14-601.1>
- Lloret, A., Addie, D.D., Boucraut-Baralon, C., et al.: European advisory board on cat diseases. Cytaruxoonosis in cats: ABCD guidelines on prevention and management. *J. Feline Med. Surg.* **17**, 637–641 (2015)
- Lopes de Carvalho, I., Nuncio, M.S.: Laboratory diagnosis of Lyme borreliosis at the Portuguese National Institute of Health (1990–2004). *Euro. Surveill.* **11**(10), 257–260 (2006)
- Lopes de Carvalho, I., Toledo, A., Carvalho, C.L., et al.: Francisella species in ticks and animals, Iberian Peninsula. *Ticks Tick Borne Dis.* **7**(1), 159–165 (2016). <https://doi.org/10.1016/j.ttbdis.2015.10.009>
- Maia, C., Almeida, B., Coimbra, M., et al.: Bacterial and protozoal agents of canine vector-borne diseases in the blood of domestic and stray dogs from southern Portugal. *Parasites Vectors.* **8**, 138 (2015). <https://doi.org/10.1186/s13071-015-0759-8>
- Matos, M., Alho, A.M., Owen, S.P., et al.: Parasite control practices in companion animals: a survey of dog and cat owners. *Prev. Vet. Med.* **122**, 174–180 (2015)
- Matuschka, F.R., Klug, B., Schinkel, T.W., et al.: Diversity of European Lyme disease spirochetes at the southern margin of their range. *Appl. Environ. Microbiol.* **64**, 1980–1982 (1998)
- Mellor, P.S., Boned, J., Hamblin, C., Graham, S.: Isolations of African horse sickness virus from vector insects made during the 1988 epizootic in Spain. *Epidemiol. Infect.* **105**, 447–454 (1990)
- Milhano, N., de Carvalho, I.L., Alves, A.S., et al.: Coinfections of *Rickettsia slovacica* and *Rickettsia helvetica* with *Borrelia lusitaniae* in ticks collected in a Safari Park, Portugal. *Ticks Tick Borne Dis.* **1**(4), 172–177 (2010). <https://doi.org/10.1016/j.ttbdis.2010.09.003>
- Norte, A.C., de Carvalho, I., Ramos, J.A., et al.: Diversity and seasonal patterns of ticks parasitizing wild birds in western Portugal. *Exp. Appl. Acarol.* **58**, 327–339 (2012)
- Nuncio, M.S., Péter, O., Alves, et al.: Isolamento e caracterização de borrelias de *Ixodes ricinus* L. em Portugal. *Separata Revista Portuguesa Doenças Infecciosas* **16**, 175–179 (1993)
- Nunes, M., Parreira, R., Lopes, N., et al.: Molecular identification of *Borrelia miyamotoi* in *Ixodes ricinus* from Portugal. *Vector Borne Zoonotic Dis.* **15**, 515–517 (2015)
- Nunes, M., Parreira, R., Maia, C., et al.: Molecular identification of *Borrelia* genus in questing hard ticks from Portugal: phylogenetic characterization of two novel relapsing fever-like *Borrelia* sp. *Infect. Genet. Evol.* **40**, 266–274 (2016)
- Office International des Epizooties – Animal Diseases: Bluetongue (2018). <http://www.oie.int/en/animal-health-in-the-world/animal-diseases/bluetongue/>. Accessed 17 Nov 2018
- Otranto, D., Dantas-Torres, F., Brianti, E., et al.: Vector-borne helminths of dogs and humans in Europe. *Parasites Vectors* **6**, 16 (2013)
- Pereira, A., Figueira, L., Nunes, M., et al.: Multiple Phlebovirus (Bunyaviridae) genetic groups detected in *Rhipicephalus*, *Hyalomma* and *Dermacentor* ticks from southern Portugal. *Ticks Tick Borne Dis.* **8**, 45–52 (2017)
- Portillo, A., de Sousa, R., Santibáñez, S., et al.: Guidelines for the detection of *Rickettsia* spp. *Vector-Borne Zoonotic Dis.* **17**(1), 23–32 (2017). Guidelines Issue, Edit. Stephen Higgs
- Price, R.D., Hellenthal, R.A., Palma, R.L.: World checklist of chewing lice with host associations and keys to families and genera. In: Price, R.D., Hellenthal, R.A., Palma, R.L., et al. (eds.) *The Chewing Lice: World Checklist and Biological Overview*, pp. 1–448. Illinois Natural History Survey Special Publication, Champaign (2003)

- Ramilo, D., Garros, C., Mathieu, B., et al.: Description of *Culicoides paradoxalis* sp. nov. from France and Portugal (Diptera: Ceratopogonidae). *Zootaxa* **3745**, 243–256 (2013)
- Ramilo, D.W.: Phenotypic and genetic characterization of *Culicoides* (Diptera: Ceratopogonidae) in Portugal and comparison of the effect of pyrethroid insecticides in their control. Ph.D. thesis in Veterinary Sciences, Animal Health. Faculty of Veterinary Medicine, University of Lisbon, p. 276 (2016). <http://hdl.handle.net/10400.5/11331>
- Ramilo, D.W., Alexandre-Pires, G., Santos, M., Pereira da Fonseca, I.: Insects of *Culicoides* genus in Portugal and their relevance in Veterinary Medicine. *Revista Portuguesa de Ciências Veterinárias* **113**, 10–17 (2018)
- Ramilo, D.W., Diaz, S., Pereira da Fonseca, I., et al.: First report of 13 species of *Culicoides* (Diptera: Ceratopogonidae) in mainland Portugal and Azores by morphological and molecular characterization. *PLoS One* **7**, e34896 (2012)
- Ramilo, D.W., Nunes, T., Madeira, S., et al.: Geographical distribution of *Culicoides* (Diptera: Ceratopogonidae) in mainland Portugal: presence/absence modelling of vector and potential vector species. *PLoS One* **12**, e0180606 (2017)
- REVIVE. Rede de Vigilância de Vectores - Culicídeos e Ixodídeos. Instituto Nacional de Saúde Doutor Ricardo Jorge, Abril 2015, Lisboa, pp. 29–48 (2014)
- REVIVE. Rede de Vigilância de Vectores - Culicídeos e Ixodídeos. Instituto Nacional de Saúde Doutor Ricardo Jorge, Abril 2018, Lisboa, pp. 29–49 (2017)
- Ribeiro, R., Wilson, A.J., Nunes, T., et al.: Spatial and temporal distribution of *Culicoides* species in mainland Portugal (2005–2010). Results of the Portuguese Entomological Surveillance Program. *PLoS One* **10**, e0124019 (2015)
- Samuel, W.M., Williams, E.S., Rippin, A.B.: Infestations of *Piagetia peralis* (Mallophaga: Menoponidae) on juvenile white pelicans. *Can. J. Zool.* **60**, 951–953 (1982). <https://doi.org/10.1139/z82-130>
- Sanches, G.S., Couto, J., Silva-Pedrosa, R., et al.: Molecular heterogeneity of *Rhipicephalus sanguineus* sensu lato and screening for *Ehrlichia canis* in mainland Portugal. *Ticks Tick Borne Dis.* **9**(6), 1383–1390 (2018). <https://doi.org/10.1016/j.ttbdis.2018.06.002>
- Santos, A.S., de Bruin, A., Veloso, A.R., et al.: Detection of *Anaplasma phagocytophilum*, *Candidatus Neoehrlichia* sp., *Coxiella burnetii* and *Rickettsia* spp. in questing ticks from a recreational park, Portugal. *Ticks Tick Borne Dis.* **9**(6), 1555–1564 (2018). <https://doi.org/10.1016/j.ttbdis.2018.07.010>
- Santos, A.S., Alexandre, N., Sousa, R., et al.: Serological and molecular survey of *Anaplasma* species infection in dogs with suspected tickborne disease in Portugal. *Vet Rec.* **164**(6), 168–171 (2009)
- Santos, A.S., Santos-Silva, M.M., Almeida, V.C., et al.: Detection of *Anaplasma phagocytophilum* DNA in *Ixodes* ticks (Acari: Ixodidae) from Madeira Island and Setubal District, mainland Portugal. *Emerg. Infect. Dis.* **10**(9), 1643–1648 (2004)
- Santos-Silva, M.M., Beati, L., Santos, A.S., et al.: The hard-tick fauna of mainland Portugal (Acari: Ixodidae): an update on geographical distribution and known associations with hosts and pathogens. *Exp. Appl. Acarol.* **55**(1), 85–121 (2011). <https://doi.org/10.1007/s10493-011-9440-x>
- Santos-Silva, M.M., Melo, P., Santos, N., et al.: PCR screening of tick-borne agents in sensitive conservation areas, Southeast Portugal. *Mol. Cell. Probes* **31**, 42–45 (2017). <https://doi.org/10.1016/j.mcp.2016.11.005>
- Santos, M., Soares, R., Costa, P., Amaro, A., Inácio, J., Gomes, J.: Revisiting the Tams1-encoding gene as a species-specific target for the molecular detection of *Theileria annulata* in bovine blood samples. *Ticks Tick Borne Dis.* **4**(1–2), 72–77 (2013). <https://doi.org/10.1016/j.ttbdis.2012.07.006>

- Saxena, A.K., Agarwal, G.P., Chandra, S., Singh, O.P.: Pathogenic involvement of Mallophaga. *Zeitschrift für Angewandte Entomologie* **99**, 294–301 (1985). <https://doi.org/10.1111/j.1439-0418.1985.tb01991.x>
- Seegar, W.S., Schiller, E.L., Sladen, W.J.L., Trpis, M.: A mallophaga, *Trinoton anserinum*, as a cyclodevelopmental vector for a heartworm parasite of waterfowl. *Science* **194**, 739–741 (1976). <https://doi.org/10.1126/science.982042>
- Shiels, B., McDougall, C., Tait, A., Brown, C.G.: Antigenic diversity of *Theileria annulata* macroschizonts. *Vet. Parasitol.* **21**(1), 1–10 (1986)
- Silaghi, C., Santos, A.S., Gomes, J., Christova, I., Matei, I.A., Walder, G., Domingos, A., Bell-Sakyi, L., Sprong, H., von Loewenich, F.D., Oteo, J.A., de la Fuente, J., Dumler, J.S.: Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. *Vector Borne Zoonotic Dis.* **17**(1), 12–22 (2017). <https://doi.org/10.1089/vbz.2016.1960>
- Silva, M.G., Marques, P.X., Oliva, A.: Detection of *Babesia* and *Theileria* species infection in cattle from Portugal using a reverse line blotting method. *Vet. Parasitol.* **174**(3–4), 199–205 (2010)
- Simões, P.B., Cardoso, L., Araújo, M., et al.: Babesiosis due to the canine *Babesia microti*-like small piroplasm in dogs—first report from Portugal and possible vertical transmission. *Parasites Vectors* **4**, 50 (2011)
- Stenberg Lewerin, S., Hallgren, C., Miezewska, K., et al.: Infection with bluetongue virus serotype 8 in Sweden in 2008. *Vet. Rec.* **167**, 165–170 (2010)
- Tendeiro, J.: Estudos sobre malófagos: revisão monográfica do género *Columbicola* Ewing (*Ischnocera*, *Phloptera*). *Memórias da Junta de Investigações do Ultramar*, 2ª série, Nº 32, Lisboa (1962)
- Tomás, A., Palma, R.L., Rebelo, M.T., da Fonseca, I.P.: Chewing lice (Phthiraptera) from wild birds in southern Portugal. *Parasitol. Int.* **65**, 295–301 (2016). <https://doi.org/10.1016/j.parint.2016.02.007>
- Tompkins, D.M., Jones, T., Clayton, D.H.: Effect of vertically transmitted ectoparasites on the reproductive success of swifts (*Apus apus*). *Funct. Ecol.* **10**, 733 (1996). <https://doi.org/10.2307/2390508>
- Traversa, D., Di Cesare, A., Conboy, G.: Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. *Parasites Vectors* **3**, 62 (2010)
- Vanbinst, T., Vandebussche, F., Vandemeulebroucke, E., et al.: Bluetongue virus detection by real-time RT-PCR in *Culicoides* captured during the 2006 epizootic in Belgium and development of an internal control. *Transbound. Emerg. Dis.* **56**, 170–177 (2009)
- Weir, W., Ben-Miled, L., Karageç, T., et al.: Genetic exchange and sub-structuring in *Theileria annulata* populations. *Mol. Biochem. Parasitol.* **154**(2), 170–180 (2007)
- Weir, W., Karageç, T., Gharbi, M., Simuunza, M., Aypak, S., Aysul, N., Darghouth, M.A., Shiels, B., Tait, A. Population diversity and multiplicity of infection in *Theileria annulata*. *Int. J. Parasitol.* **41**(2), 193–203 (2011). <https://doi.org/10.1016/j.ijpara.2010.08.004>
- Wilson, A.J., Mellor, P.S.: Bluetongue in Europe: past, present and future. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**, 2669–2681 (2009)
- Yusuf, J.J.: Review on Bovine Babesiosis and its economical importance. *J. Vet. Med. Res.* **4**(5), 1090 (2017)