Emerging Hyalomma lusitanicum: From identification to vectorial role and integrated control

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Running title: Emerging *Hyalomma lusitanicum*: a review

Title: Emerging *Hyalomma lusitanicum*: from identification to vectorial role and integrated control

F. Valcárcel[1], L. Elhachimi[2], M. Vilá[3], L. Tomassone[4], M. Sánchez[1,5,6], Selles SMA[7,8], Kouidri M.[9], M. G. González[1,5], R. Martín-Hernández[10], Á. Valcárcel[11,16], N. Fernández[6], J. M. Tercero[5], J. Sanchis[12], J. Bellido-Blasco[13], A. González-Coloma[14], A. S. Olmeda[15].

1 Grupo de Parasitología Animal, Departamento de Reproducción Animal, INIA-CSIC, 28040 Madrid, Spain; valcarcel.felix@inia.csic.es (F.V.)
2 Département de parasitologie et de Santé Publique, Institut Agronomique et Vétérinaire Hassan II, B.P. 6202, Rabat; Marocco; latifa.elhachimi@gmail.com (L.E.)
3 Grupo de Investigación COPAR (GI-2120; USC), Departamento de Patoloxia Animal, Facultade de Veterinaria, Universidade de Santiago de Compostela. 27002-Lugo (España), maria.vila.pena@gmail.com (M.V.)
4 Department of Veterinary Sciences, University of Turin, Grugliasco (Turin), Italy; laura.tomassone@unito.it (L.T.)
5 Villamagna S.A., Finca ''La Garganta'', 14440 Villanueva de Córdoba, Spain; marta.gonzalez@inia.es (M.G.G.); maria2985@hotmail.com (M.S.); jmtercero@fincalagarganta.com (J.M.T.)
6 Facultad de Veterinaria, Universidad Alfonso X El Sabio (UAX), Avda. Universidad, 1, 28691 Villanueva de la Cañada, Madrid, Spain; mrsnchz@uax.es (M.S.); nfernpat@uax.es (N.F.)
7 Institute of Veterinary Sciences, University of Tiaret, 14000 Tiaret, Algeria; mohammedammarselles@yahoo.fr (S.S.M.A,)
8 Laboratory of Research on Local Animal Products, University of Tiaret, 14000 Tiaret, Algeria; nuider10@yahoo.fr (S.S.M.A,)
9 Laboratory of Farm Animal Products, University of Tiaret, 14000 Tiaret, Algeria; mokhtariakouidri@yahoo.fr (M.K.)
10 Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), IRIAF – Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Marchamalo, 19180, Spain. Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT – ESF/EC-FSE), Fundación Parque Científico y Tecnológico de Castilla–La Mancha, Albacete, 02006, Spain; rmhernandez@jccm.es (R. M.)

11 Lokimica S.L., c/ Valdemorillo, 51b, 28925 Alcorcón, Madrid, Spain; angelavalcarcelolmeda@gmail.com (A. V.)
12 Universidad de la República, Facultad de Veterinaria, CENUR Litoral Norte, Uruguay; sanchisjaime@gmail.com (J. S.)
13 Sección de Epidemiología, Centro de Salud Pública de Castelló, CIBER-ESP. Universitat Jaume I (UJI), Castelló, Spain; bellido_jua@gva.es (J. B.)
14 Instituto de Ciencias Agrarias (ICA), CSIC, Madrid, Spain. azu@ica.csic.es (A. G)
15 Departamento de Sanidad Animal, Facultad de Veterinaria, UCM, 28040 Madrid, Spain. angeles@ucm.es (A. S. O)
16 Veterinary Pathobiology section, University College Dublín, Belfield, Dublin 4 Ireland. angela.valcarcelolmeda@ucdconnect.ie

* **Corresponding author:** Félix Valcárcel, Grupo de Parasitología Animal, Departamento de Reproducción Animal, INIA-CSIC, 28040 Madrid, Spain valcarcel.felix@inia.csic.es ; Tel.: +34-91-3474046

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Abstract

In the Mediterranean basin, the tick species *Hyalomma lusitanicum* Koch stands out among other species of the *Hyalomma* genus due to its wide distribution and there is great concern about its potential role as a vector and/or reservoir and its continuous expansion to new areas because of climate warming and human and other animal movements.

This review aims to consolidate all the information on *Hyalomma lusitanicum* including taxonomy and evolution, morphological and molecular identification, life cycle, sampling methods, rearing under laboratory conditions, ecology, hosts, geographical distribution, seasonality, vector role, and control methods. Availability of adequate data is extremely relevant to develop the appropriate control strategies in areas where this tick is currently distributed, as well as in new areas where it could become established in the near future.

Key words

Acari, Ixodidae, life cycle, ecology, laboratory colonies, sampling, seasonality

Introduction

Ticks are a large group of blood-feeding arthropods belonging to the following families: Deinocrotonidae (2 species), Nuttalliellidae (1 species), Argasidae (soft ticks, 186 species) and Ixodidae (hard ticks, 720 species) (Barker and Murrell, 2004; Guglielmone et al., 2010, 2014; Peñalver et al., 2017; Chitimia-Dobler et al., 2022) usually identified according to morphological features although their phylogeny can be determined by molecular characterization (Rees et al., 2003; Abdullah et al., 2016, 2018; Kamani et al., 2017; Schulz et al., 2020).

Hard ticks may feed on many hosts and survive in different habitats, climates and conditions depending on their development stage, species, host abundance and other factors. In general, abundant and diverse information on life cycle characteristics such as geographical distribution, hosts and developmental stages are available, however sometimes this information is difficult to access (Guglielmone et al., 2014). This is the case with *Hyalomma*, a common genus of hard ticks found in southern Europe, Africa and Asia. Its Latin name means glass (*hyalos*) eyes (*omma*). These distinctive eyes along with their banded legs and large size are characteristic of this genus. Some species of *Hyalomma* are adapted to desert environments and have managed to survive in harsh conditions with limited food resources during times of major environmental changes worldwide, which may contribute significantly to the wide-ranging distribution and diversity of the species (Sands et al., 2017). Identification of the *Hyalomma* species remains difficult owing to their genetic and morphological variations, which gave rise to the recent redescription of the morphology of some species (Apanaskevich and Horak, 2006, 2007, 2008, 2009; Apanaskevich et al., 2008, 2010). Within this genus, *Hyalomma lusitanicum* Koch, stands out for its wide distribution and the role it plays in the transmission of several pathogens of medical and veterinary importance.

This review aims to consolidate information on *Hyalomma lusitanicum* given the possible establishment of this hard tick species in new areas in the near future.

Taxonomy and evolution

The evolutionary history of ticks developing together with their hosts has been well recognized historically. However, more recent phylogenetic studies have stressed the important roles played by ecology and habitat in this association (Klompen et al., 1996b). According to Hoogstraal and Aeschlimann (1982) ticks originated in the late Palaeozoic (approximately 225 million years ago), as obligate parasites of reptiles and gave rise to the four families known today (Deinocrotonidae, Argasidae, Ixodidae and Nuttalliellidae). Each tick family comprises different subfamilies, genera and species that are part of a classification still evolving and based on morphological identification and phylogenetic interpretation using molecular methods. This section outlines the different theories on the evolutionary history of ticks leading to the current classification of *H. lusitanicum* (Table 1).

Table 1.

According to Hoogstraal (1985), the subfamilies of Ixodidae diversified based on modifications of the mouth and coxae structure associated with adaptation to their hosts. Hyalomminae is a subfamily from the Metastriata group and apparently was one of the subfamiles best adapted to survive hostile environments and low host density during the Cretaceous (Peñalver et al., 2017), the period during which a mass extinction of animals took place. However, in terms of their morphological characteristics, host relationships and biogeography, and based on genetic and molecular advances, the evolutionary history of ticks and their systematics continues to develop (Durden and Beati, 2014).

The taxonomy of *H. lusitanicum* has been modified throughout history since the time adults were first described as a species by Koch (1844) and larvae and nymphs by Sénevet (1925, 1928). All stages were subsequently redescribed (Koch, 1844, 1850; Tendeiro, 1955; Hoogstraal and Kaiser, 1959; Feldman-Muhsam, 1962; Senevet and Ripert, 1964; Perez-Eid and Cabrita, 2003; Pérez-Eid, 2007; Apanaskevich et al., 2008; Estrada-Peña et al., 2017). It has been reported in the literature as *H. lusitanicum*, *H. (Hyalomma) lusitanicum* and *H. (Euhyalomma) lusitanicum* (Hueli et al., 1984; Ouhelli and Pandey, 1984a; Perez-Eid and Cabrita, 2003; Walker et al., 2003; Estrada-Peña et al., 2004, 2014, 2017; Apanaskevich et al., 2008). It has also been considered as a variety of *Hyalomma aegyptium* (Neumann, 1899, 1901), a polymorphic species (Senevet, 1922; Schulze and Schlottke, 1930) and as synonymous to *Hyalomma excavatum* (Delpy, 1949; Feldman-Muhsam, 1954; Hoogstraal, 1956). Today it is accepted as a distinct species: *Hyalomma lusitanicum* (Guglielmone et al., 2020).

Hyalomma lusitanicum is a Palearctic species restricted to countries of the western part of the Mediterranean subregion: France, Italy, Portugal, Spain (including the Canary Islands), Algeria and Morocco. However, it is slowly expanding northwards to areas in the northwest of France, the United Kingdom (Starkoff, 1958; Morel, 1959; Feldman-Muhsam, 1962; Tendeiro, 1962; Manilla, 1998; Apanaskevich et al., 2008; Estrada-Peña et al., 2017; Guglielmone et al., 2020; Valcárcel et al., 2020), and has occasionally been reported in other countries such as Egypt (Okely et al., 2022).

Morphological identification

Hyalomma spp. adults are medium-large-sized ticks (Wall and Shearer, 2001) with a pair of spiracles on each side of the idiosoma below the fourth coxa (in nymphs and adults). The basis capituli is rectangular and the large mouthparts project towards the anterior margin of the body. The highly convex bulging eyes have a circular groove surrounding the eye socket. Legs are characterised by generally pale coloured rings at the end of most segments. Finally, in males, a posterior Y-shaped groove surrounds the anus, showing well-developed ventral plates (Gil Collado et al., 1979; Walker et al., 2003; Apanaskevich et al., 2008; Apanaskevich and Horak, 2008; Estrada-Peña et al., 2014; Sonenshine and Roe, 2014) (Figure 1).

Koch (1850) described *H. lusitanicum* (from Portugal) as almost identical in form to *Hyalomma hispanum* Koch (from Spain) as wine-red ticks, with a narrow yellowish-white band; legs rust-red, dotted with brownish-red, the tips of the limbs are broad light yellow, also dotted with red. At present, it is presumed that both are the same species (Delpy, 1949). In Koch´s *H. lusitanicum* description (1850) the male is characterised by a coarsely dotted cervical shield that protrudes in front; the abdomen wrinkled dark wine-red, the sides dull yellowish. The base of the capitulum is quadrangular, curved at the posterior margin, with the usual longitudinal protuberances on the sides. The palps and hypostome are rather long, the former somewhat broad, the upper part somewhat sunken. On the lower part of the body are the same ventral shields. The shield is wine-red, the anterior part darker and the posterior part of the body unevenly spotted with black, the border running around it white-yellow and very thin. Ventrally it is yellow, with rusty red spots on the shield. The gnathosoma is light reddish, as are the legs, although the latter are splashed with darker colour; the margin of the limbs is broad and whitish-yellow, also with dark reddish spots.

The female is more elongated and has deeper furrows on the shield, which is also more dotted. The shield is yellow, the lateral parts brown behind, the central part yellow-red behind and the curved grooves are deep brown. The idiosome is strongly wrinkled and has deep longitudinal folds in the usual position. It is darker wine-red, with notches on the marginal scutum and a yellowish border on the sides. Ventrally is yellowish red. All the rest of the characteristics of the female are the same as the male.

Our unpublished data show significant variations in the size and weight of adult *H. lusitanicum* in central Spain, with females being slightly longer (n= 30, mean: 4,832.83 mm, SD: 757.67, min: 1,045.16, max: 5,499.76) than males (n= 30, 4,656.09 mm, SD: 260.42, min: 4087.84, max: 5055.26) and significantly heavier (females: n= 100, mean: 6.24 mg, SD: 1.908, min: 2, max: 11; males: n= 100, mean: 4.54 mg, SD: 1.520, min: 2, max: 9) (t= 6.969 $p < 0.01$, 198 DF). They are similar in size to other *Hyalomma* species reported in the literature (Tendeiro, 1955; Apanaskevich et al., 2008, 2010; Apanaskevich and Horak, 2006, 2007, 2008 a,b, 2009; Torres de Almeida). Table 2 shows the comparative length and width of the scutum/conscutum of different *Hyalomma* species, and Tables 3 to 5 summarize the morphological characteristics of the three developmental stages of *H. lusitanicum* described in the literature.

Table 2.

Table 3.

Table 4.

Table 5.

Molecular identification

Accurate identification of *Hyalomma* species is an essential prerequisite to understanding the eco-epidemiology of vector-transmitted diseases and identifying vector and pathogen reservoir species. In this connection, taxonomists are still debating the advantages and disadvantages of morphological versus molecular identification. Molecular identification may be useful when its morphological counterpart is difficult, for instance in the case of immature stages, damaged specimens, blood-fed or semi-blood-fed ticks and species with a similar morphology. A recent study conducted by fourteen teams of tick experts concluded that *H. lusitanicum* was the second most frequently misidentified tick species, especially in areas where this tick species is not found (Estrada-Peña et al., 2017). Hence, molecular tools can improve the accuracy of identification of this tick species.

Other advantages of molecular tools include: a description of the host-pathogen association, data on the microbiome and co-infections with other pathogens, and detection of possible tick borne disease transmission risk scenarios. These tools can also help in gaining a deeper understanding of the transmission cycles of any *H. lusitanicum* transmitted pathogen and associated dynamics and, consequently, aid in the design and implementation of sustainable, effective and adapted control strategies (Hernández-Andrade et al., 2019). Lastly, molecular tools are extremely helpful in clarifying the taxonomic status of problematic Ixodidae taxa (Krčmar et al., 2022).

The success of molecular identification, however, depends on the accurate morphological identification of reference specimens before uploading their sequences to genetic databases, as such sequences will then be used for further classification. In addition, the correct assignment of genetic information is necessary for the molecular identification of a species based on gene sequencing, genus-specific PCR design or metabarcoding techniques (Beati et al., 2013).

Most molecular techniques are based on the amplification of ribosomal genes. Arthropod rDNA contains genes for 18S, 5,8S and 28S rRNA, two internal transcribed spacers (ITS 1 and ITS 2) flanking the 5,8S gene and an intergenic spacer (McLain et al., 1995). However, consultation with GenBank at the time of writing this article showed that most of the rRNA of *H. lusitanicum* sequences (62.5%) are named 16S instead 18S. Inconsistencies also were found when comparing the size of the ribosomal gene sequences uploaded to Genbank and therefore must be reviewed. For other genes (e.g., COI or histone 3), only partial information is available to date. A combination of three DNA markers is recommended to prevent misidentification (Lv et al., 2014).

General description of the life cycle

Hyalomma lusitanicum is a three-host tick; after each feeding period, ticks leave the host to moult, in the case of immature stages, or to lay eggs in the case of females (Estrada-Peña et al., 2004; Jongejan and Uilenberg, 2005; Sonenshine, 2009). Although *H. lusitanicum* may feed on a wide range of hosts (Cordero del Campillo et al., 1980; García Fernández and Hueli, 1984; Dias, 1994; Perez-Eid and Cabrita, 2003; Monsonis, 2016; Valcárcel et al., 2020) they are usually classified as a ditropic species because immature stages feed mainly on rabbits and adults on wild ungulates (Ouhelli, 1994; Manilla, 1998; Ruiz-Fons et al., 2006; Apanaskevich et al., 2008; Guglielmone et al., 2010). However, based on frequent detection of immature stages feeding on red deer (Valcárcel et al., 2016b), it was suggested that *H. lusitanicum* should be also classified as a telotropic species (Valcárcel et al., 2020), i.e. characterized by the ability of immature individuals to feed on the same hosts as adults (Walker et al., 2003).

The total length of the *H. lusitanicum* life cycle is 101-193 days under controlled conditions, such as temperature, humidity and lighting period [22 ºC, 80% relative humidity (RH), natural photoperiod] (Ouhelli and Pandey, 1984a; Cota Guajardo, 2015). However, unfed tick-life stages can survive for years in the soil with an exceptionally low metabolic rate (Lighton and Fielden, 1995), mainly owing to diapause. Diapause can be morphogenetic (delay in the metamorphosis of fed larvae and nymphs, ovogenetic in gravid females and embryogenetic in the egg) or behavioral (interruption of host-seeking strategy in unfed ticks) (Belozerov, 1982). When conditions are suitable, the oviposition period, which is 15-26 days long, occurs 8-47 days after female engorgement. However, morphogenetic diapause occurs when engorged females delay egg-laying until spring if they are collected from animals in the autumn or when females are artificially engorged in late summer and kept under laboratory conditions (22 ºC, 80% RH) (González et al., 2017).

Unfed *H. lusitanicum* adults typically remain hidden in the soil during the colder months of the year, but may start searching for a host when the daily temperature occasionally rises above 20°C, even in winter (Valcárcel et al., 2016a). We have not observed this behavior in immature stages and have not found references to it, but it is to be expected that they do not survive in cold conditions.

Host search

Immature stages of *H. lusitanicum* are usually endophilic, remaining in the host's burrow or shelter where contact with the host is easier. However, they may exhibit exophilic behavior if the wild rabbit population is scarce or the tick population excessive (Valcárcel et al., 2020). In contrast, adults are always exophilic giving the possibility of choosing from a wider range of hosts, but with the disadvantage of less contact, forcing them to adopt different host searching strategies. Questing is a very common host searching behavior of *H. lusitanicum* adults (Valcárcel et al., 2020, 2016a) when vegetation is 30 cm or higher. However, when vegetation is lower, adults of *H. lusitanicum* adopt a "sit and wait" strategy, remaining hidden on the ground until they detect a potential host and quickly pursue it (hunting) (Valcárcel, personal observations).

Certain stimuli, such as the release of host-derived semiochemicals (specifically kairomones), are necessary for ticks engaging in a hunting strategy to leave their microhabitat and move in the direction of their host. In the laboratory and the field, *H. lusitanicum* responds quickly to the carbon dioxide exhaled by the host, similar to other species such as *Amblyomma variegatum* Fabricius*, Rhipicephalus sanguineus* Latreille and *Ixodes ricinus* L. (McMahon and Guerin, 2002). We also have observed that hidden *H. lusitanicum* adults chase humans as soon as the latter are detected and actively climb up their extremities but have a low attachment. After ten to fifteen minutes, adults appear to lose interest and stop chasing the potential host, and most drop off without attaching; however, when humans return to the same place after 10 to 15 minutes, *H. lusitanicum* adults quickly chase after them again (Valcárcel*,* personal observation). This could be because humans are not the preferred host but, it could be done to avoid excessive water loss (Z. Vatansever, personal communication).

Feeding site, attachment, repletion, and detachment.

After finding a host and contacting its skin, ticks search for a suitable place to feed (Marquez and Guiguen, 1992; Kiffner et al., 2011), usually areas that the host itself cannot easily access. Skin thickness and hair or fur density are also determining factors. *Hyalomma lusitanicum* can attach to any part of a host body but it prefers areas with little hair such as ears, belly or around the eyes (in wild rabbits or small birds) or on the hindquarters, inner thigh, anus or udders (in ungulates) (Valcárcel et al., 2020).

In some species (e.g. *Rhipicephalus (Boophilus) microplus* Canestrini or *Hyalomma marginatum* Koch) feeding placement is facilitated by the emission of aggregation and sexual recognition pheromones (Diehl et al., 1982; Estrada-Peña and de la Fuente, 2014), the reason why congregations of these ticks often appear on hosts. However, there is no descriptions of any pheromone types in *H.*

lusitanicum (Starkoff, 1958; Ouhelli, 1994; Lighton and Fielden, 1995). Congregation on hosts is rarely observed in *H. lusitanicum*, either in cases of high natural infestation or in artificial feeding assays.

The feeding process in adults occurs in two phases (Hoogstraal, 1985), a slow phase over 4-8 days that includes copulation, and a final rapid phase lasting only 24 hours during which the tick reaches its maximum weight in order to continue its biological cycle. In artificial feeding studies, we have observed that during the preparatory phase *H. lusitanicum* females remain in a vertical position for one or two days with the hypostome crossing the silicone membrane and the palps held outside the attachment point. Later, the idiosome adopts a position almost parallel to the silicone membrane surface and the slow phase begins. This may last from four to five days under artificial feeding conditions and extend to three to four weeks in experimental infestation of sheep before starting the rapid phase during which they engorge in approximately one to two days (Cota Guajardo, 2015; González et al., 2017).

Mating, oviposition, hatching, feeding and moulting

Once on the host, as most genera, partially fed *H. lusitanicum* females remain there until fertilization by the male (Diehl et al., 1982). The weight of artificially engorged *H. lusitanicum* females varies greatly (min: 52 mg, max: 600 mg), heavier in spring-summer than in autumn-winter but the average weight (274 mg) (González et al., 2017) is lower than engorged females fed on calves (543 mg) (Ouhelli and Pandey, 1984b). Nevertheless, low-weight females can still lay eggs (González et al., 2020, 2017). The following definitions of the life cycle phases are based on Troughton and Levin (2007), González et al. (2016) and Valcárcel et al. (2020): Feeding: number of days from placement on the host until drop-off; moulting: number of days from drop-off to ecdysis; gestation/pre-oviposition: period from female drop-off to the start of egg laying; incubation: period from the first egg is laid until approximately 50% of hatching of larvae; and oviposition: period when the engorged female is laying eggs.

The gestation/pre-oviposition periods in *H. lusitanicum* are shorter in spring-summer in both artificial and animal feeding than in autumn-winter (Ouhelli and Pandey, 1984b; González et al., 2017). Gestation/pre-oviposition in *H. lusitanicum* can vary greatly from 8 to 47 days. The duration of oviposition and the number of eggs laid (629–14,519 eggs per engorged female, Valcárcel et al., 2020) depend on the host species on which the females were fed, days of feeding and weight attained (Diehl et al., 1982), temperature and relative humidity (Ouhelli and Pandey, 1984a; Ouhelli, 1994; Cota Guajardo, 2015; Elhachimi et al., 2021), which, in turn, typically depend on the season and the host or system. Oviposition from 15 to 26 days and takes place in a single step, depleting all the reserves of the female, which dies at the end of the process. The female's body partially protects the egg mass and eggs that are exposed to the environment may not hatch and also serve as protection for the rest of the egg mass (Gállego-Berenguer, 2007).

After a short period of cuticle hardening in the soil (minimum 8 days) *H. lusitanicum* larvae are ready to start looking for a host and when they find one; they can finish feeding in less than a week and moult into nymphs in about two weeks (Valcárcel et al., 2020). We have no data on cuticle hardening of nymphs and females, which may finish feeding between one and two weeks or between one and four weeks, respectively (Valcárcel et al., 2020).

Moulting periods (from drop off to ecdysis) of larvae to nymphs and nymphs to adults are slightly longer than the respective feeding period. However, in nature these periods may vary due to environmental conditions (see below for the influence of abiotic parameters). Photoperiod activates or delays moulting until favourable conditions prevail (Estrada-Peña and de la Fuente, 2014).

Sampling on vegetation

An abundance of ticks on vegetation is indicative of host search activity and is affected by various environmental and physiological factors. *Hyalomma lusitanicum* is a three-host tick, therefore sampling can be performed both on vegetation and on hosts. Hand collection of questing or hunting *H. lusitanicum* adults can easily be done from vegetation in certain hotspot areas (Valcárcel et al., 2020). However, to determine their abundance or for comparison purposes, it is better to employ standard methods such as dragging (passing a drag over ground level vegetation) or flagging (waving a flag over and through higher vegetation), although the number of ticks collected varies greatly in both methods. While some authors report a similar effectiveness using both dragging and flagging techniques to collect *Ixodes scapularis* Say nymphs (Rulison et al., 2013), we share the opinion of other authors who point out that collection effectiveness of these techniques varies according to the type and height of vegetation, season, tick stage and species (Tack et al., 2011; Mihalca et al., 2012; Dantas-Torres et al., 2013; Valcárcel et al., 2016a). In the Mediterranean environment, meadows are not frequent, but there are abundant areas of scrubland with vegetation that can vary from very short to half a metre or more in small areas, so both flagging and dragging can be used alternatively when collecting *H. lusitanicum* samples. *Hyalomma lusitanicum* can be collected where there is no vegetation or where vegetation is short, medium or tall (or any combination thereof) (Valcárcel et al., 2016a), probably due to the dual ability of adults to quest and hunt. In any case, limitations of these techniques include adverse weather conditions (wind, rain or snow), and certain types of vegetation cover that can make specimen collecting difficult (Dantas-Torres et al., 2013). Some authors have described a protocol based on line transects and sampling able to generate robust data on tick population biology (Salomon et al., 2020). In our opinion, this method could not be considered a gold standard for *H. lusitanicum* (or any other tick species) because the distribution of ticks in the soil is not uniform, but at least it is a method that allows comparison between different studies. In this sense, the size of the drag/flag is an important factor that varies according to the latest studies (Cuadrado-Matías et al., 2023). Some authors have found that dragging and flagging are equally effective (Rulison et al., 2013). Thus, to collect *H. lusitanicum* in open areas with no or short vegetation, where vegetation does not limit dragging, we use a large drag (surface area: 140 x 200 cm) with a basal axis and a rope attached, but a smaller flag (surface area: 100 x 150-200 cm), which is easier to use where vegetation is tall and dense (Valcárcel et al., 2020, 2016a).

Dry ice traps, carbon dioxide traps, activators and/or attractants such as kairomones and pheromones, can be used to enhance capture methods in many tick species (Carroll, 2002; Falco and Fish, 1992; Ginsberg and Ewing, 1989; Maranga et al., 2003; Sonenshine, 2006). To the best of our knowledge, pheromones have never been used to capture *H. lusitanicum.* CO_2 application on the flag by means of “a fine silicone rubber hose network which dispersed the

CO_2 in the shaft" improves the collection of some species such as *I. ricinus* (Gherman et al., 2012). Likewise, the use of CO_2 from a commercial fire extinguisher directly on the ground and vegetation facilitates the manual collection of *Hyalomma* adults (Alexei Pace, personal communication). In an endemic area, during the peak in host seeking *H. lusitanicum*, we observed no attraction when dry ice was placed directly on the ground or when CO_2 was applied to the flag using a commercial fire extinguisher (unpublished data).

Sampling on hosts

Some qualitative data such as infestation prevalence (percentage of animals with one or more ticks), can be obtained by direct inspection and manual tick collection and can be performed quickly while avoiding host animal welfare issues. Nevertheless, it is insufficient to determine quantitative data such as tick load. Sedation allows for prolonged inspection, allowing for the collection of higher numbers of ticks on anaesthetized than non-anaesthetized domestic or wild hosts (Wilson, 1994). In fact, many ticks may go undetected, especially in long-haired animals or when the hosts are infested by immature ticks. Examination of a small area of skin could be useful when tick location is well defined (Wilson, 1994). However, as *H. lusitanicum* may be found on any part of the host body, this method does not usually provide accurate tick burden data.

A post-mortem examination, when possible, provides more precise data regarding quantitative parameters such as the magnitude of the infestation (number of ticks per host). It is important to note that immature *H. lusitanicum* detach from the carcass after a short period while adults remain on the carcass for at least one to two days before detaching. Therefore, to avoid loss of tick samples, the carcass should be examined as soon as possible post-mortem and collection should continue for two days. In our experience, the best *H. lusitanicum* collection technique is to hang small or large animals –or their skins- upside down over a recipient filled with water for 24/48 h at room temperature, to allow the ticks to detach from the host and drop into the water. The water is then filtered through gauze and ticks are recovered using tweezers and a paintbrush. As Table 6 indicates, nearly 10 times more ticks were collected using this method compared to manual collection, especially of immature individuals (unpublished data).

The collaboration of citizen scientists provides very interesting qualitative data, such as the presence of ticks in areas not usually sampled by scientists, to complement traditional sampling methods (Eisen and Eisen, 2020) and has been instrumental in the detection of *H. lusitanicum* and other *Hyalomma* species in new areas (Hansford et al., 2016, 2018; Chitimia-Dobler et al., 2019).

Tick colony feeding

Laboratory colonies provide homogenous specimens for laboratory tests and furnish very useful information about the life cycle and possible affecting factors. Tick feeding on live animals was widely performed in the past. Whole colonies could be maintained in this way under laboratory conditions resulting in valuable data on their physiology. Traditionally, experimental animals such as rats, guinea pigs, sheep and rabbits have been used for tick feeding (Levin and Schumacher, 2016). One of the most common methods consisted of attaching a small box or capsule (pill box method), usually to the animal’s back (Heyne et al., 1987). In the case of immature *H. lusitanicum* feeding on rabbits, the most standard technique is the ear bag, which is shielded from the rabbit’s movements by an Elizabethan collar (Bailey, 1960; Olmeda García, 1992; Cota Guajardo, 2015). The experimental infestation of rabbits is very useful to maintain laboratory colonies of *H. lusitanicum* because to our knowledge an in vitro feeding assay has not been developed for immature stages. Unlike they are for larvae and nymphs, rabbits are not good hosts for *H. lusitanicum* adults (unpublished data). Due to the lesions caused by adults, their natural hosts (wild or domestic ruminants) should be used. The following description refers to an experimental infestation of sheep with unfed *H. lusitanicum* adults and is based on experimental designs for adult ticks of other species on equids (Olmeda et al., 2004). Instead of an ear bag, we use a 15 x 15 cm cloth dressing fixed to the skin with an adhesive fibre (Velcro®), which allows the dressing to be removed and repositioned for daily observation. These protocols, provided in the supplementary materials, have been approved by the Ethics Committees for Animal Experimentation of INIA and by the Competent Authorities of the Community of Madrid, Spain (PROEX 193/16 “*Alimentación* in vivo *de garrapatas ixodoideas*”).

Artificial feeding

Replacing animals with an artificial tick-feeding method has been a long and arduous process. Although ticks do indeed successfully feed, the results are not completely comparable to those observed under natural feeding conditions. The weight of the females and, consequently, size are lower (Uspensky and Ioffe-Uspensky, 1999; Bonnet and Liu, 2012; González et al., 2017).

Nowadays, due to ethical and legal limitations regarding the use of experimental animals, artificial feeding is an alternative method that offers other advantages, such as standardization of results and greater reproducibility.

As far as we know the unique artificial system used to feed *H. lusitanicum* (González et al., 2017, 2020) is an adaptation of the Kröber and Guering method to feed *I. ricinus* (Kröber and Guerin, 2007). In this system, a flexible silicone membrane allows the tick to remove its mouthparts and reattach elsewhere as the previous penetration site is closed by the elastic retraction forces of the membrane. In addition to host stimuli, such as debris and hair extract, mechanical elements such as a lattice to increase ticks' foothold were added for feeding. The key factors contributing to the success of this technique are the stimuli and blood composition, which seek to reproduce a natural microhabitat in an artificial unit. Membranes of this type also have been adapted for other species such as *Hyalomma anatolicum anatolicum* Koch, *Hyalomma dromedarii* Koch, *R. sanguineus*, *I. scapularis* and *Dermacentor reticulatus* Fabricius (Fourie et al., 2013; Andrade et al., 2014; Tajeri et al., 2016; Krull et al., 2017).

Influence of abiotic factors on ecology

Tick phenology depends on microclimate and the abundance of hosts, determining tick seasonality and activity throughout the year (Estrada-Peña and de la Fuente, 2014). Tick dynamics depend on several abiotic (climatic) factors, acting mainly on the free-living stages of ticks, and biotic factors related to hosts (Randolph, 2004). Temperature regulates the development of tick stages while relative humidity, together with air saturation deficit, regulates water loss that can cause mortality (Estrada-Peña and de la Fuente, 2014). Also, soil moisture is critical for eggs and larvae of exophilic ticks (Uspensky, 2002). In species inhabiting semi-arid regions, such as *H. lusitanicum*, the timing between oviposition and the onset of the rainy season is crucial for egg survival and larval development (Knulle and Rudolph 1982).

The microclimate in vegetation layers impacts the survival, activity and development of exophilic ticks (Estrada-Peña and de la Fuente, 2014), with higher tolerance to desiccation in ticks that actively move in search of a host (Uspensky, 2002). In cold climates, temperature probably plays the most important role in regulating phenology (along with photoperiod), whereas in dry regions, water availability is the key factor (Estrada-Peña and de la Fuente, 2014). Major differences have been observed between regions in northern Spain, characterized by mild temperatures, humid conditions and a predominance of *I. ricinus* and *Haemaphysalis punctata* Canestrini & Fanzago*,* and central Spain, where higher temperatures and drier conditions favour the predominance of *H. lusitanicum* and *Dermacentor marginatus* Sulzer (Barandika et al., 2011). High soil and environmental humidity are associated with lower *H. lusitanicum* activity (Requena-García et al., 2017).

In contrast, in endophilic tick populations such as immature stages of *H. lusitanicum,* abiotic factors are related to host habitat conditions. Host burrows not only provide a refuge from predators, but also help to cope with environmental fluctuations, favouring the survival of the ticks (Gálvez-Bravo, 2011). The organization and structure of the shelter microhabitat are key for tick survival: depth, entrance width, type of soil, size, etc. (Gray et al., 2014).

Climate change also affects tick populations, both in terms of higher temperatures and periods of drought or high rainfall (Gray et al., 2009). In general, it can be assumed that tick populations will become more abundant at higher altitudes and northern latitudes as a result of global warming, which would undoubtedly have an impact on the prevalence of tick-borne pathogens (Gilbert, 2010a; Dantas-Torres, 2015). In fact, *H. lusitanicum* can now be easily collected from animals and vegetation at higher elevations than during the last few decades of the 20th century (Olmeda et al., 2019). The warming climate could have a direct effect on seasonal activity patterns (Belozerov, 1982). If temperature increase is moderate, cycle development would accelerate thus increasing activity. However, if temperatures rise more quickly, mortality would increase. This direct dependence on temperature can be observed in *H. lusitanicum* in meso-Mediterranean areas where engorged females may be collected from red deer year round in central Spain (Valcárcel et al., 2016b). This means that at least four generations of *H. lusitanicum* can be expected to initiate their cycle in the course of the year, but the length of the cycle depends heavily on the temperature during the engorgement season. Larvae hatched in spring develop quickly and adults will most likely find a host during that same season, but larvae hatched in autumn will take longer to reach the adult stage due to cold temperatures and most of these adults will have to wait until the following spring (Valcárcel et al., 2016 a,b).

In addition to the direct effect of temperature, other changes can be expected owing to indirect effects. For example, a warming climate in Central Europe could lead to a decline in spruce trees [(*Picea abies* (L.) H.Karst) (Pinales, Pinaceae)] possibly replaced by beech forests [*Fagus sylvatica* L. (Fagales, Fagaceae)], which provide a favourable microclimate for tick survival (Gray et al., 2009). Similarly, these changes would affect animals living in forests and serving as hosts for ticks.

Another example in the Mediterranean region is increasing temperatures and decreasing precipitation, which could favour the spread of *Rhipicephalus bursa Canestrini & Fanzago, Rhipicephalus turanicus* Pomerantsev et al and *H. marginatum* (Estrada-Peña and Venzal, 2007), similar to what is happening with *H. lusitanicum.* Increasing drought would have a strong impact on vegetation structure, making land tracts more open thus creating optimal conditions for exophilic tick populations, primarily of the genera *Rhipicephalus* and *Hyalomma* (Gray et al., 2009).

As already mentioned, dragging and flagging work poorly on windy days. In a seven-year study of monthly collections of adult host-seeking *H. lusitanicum,* more than 86% were collected on days with little or no wind and only 13% were collected in moderate or strong wind (Valcárcel et al., 2016a). Furthermore, host-seeking adults do not like windy days and it is impossible to collect them on such days in areas where the manual collection is otherwise very easy (Valcárcel, personal observation).

Influence of biotic factors on ecology

In addition to climate, there are two other essential parameters influencing tick life cycles: the composition and abundance of the host community. Host behavior and habitat preferences also influence the tick cycle (Dobson et al., 2011; Ginsberg et al., 2002). In the natural environment, ticks typically disperse when seeking their hosts and therefore the movement of host populations is an important factor when studying tick densities in certain areas. Field studies have shown that the habitats most frequented by hosts are the ones with the highest *H. lusitanicum* densities, whereas less frequented areas tend to have smaller or even no tick populations at all, even if environmental (climatic) conditions are suitable (González et al., 2016b; Valcárcel et al., 2016b). Therefore, the spatial structure of the landscape and its connectivity are key factors in understanding the occurrence of local tick populations (Estrada-Peña, 2008). Human-induced changes in nature have modified fauna favouring the increase of some vertebrate populations, e.g. the promotion of some game species can be the cause of the decline in the population of other animals, as in some game reserves where wild boar compete for food and displace wild rabbits, thus disturbing the ecological balance (Carpio et al., 2014).

Hyalomma lusitanicum adults are usually found on Artiodactyla (Bovidae, Cervidae, Suidae) while larvae and nymphs are commonly recovered from Lagomorpha (Leporidae) (Walker et al., 2003; Estrada-Peña et al., 2017; Guglielmone et al., 2020), which can simultaneously host a few adult ticks (González et al., 2016). Adults and nymphs have occasionally been collected from other several Mammalia orders and Galliformes (Phasianidae); adults have rarely been found on Passeriformes (Fringillidae), Strigiformes (Strigidae) or Struthioniformes (Struthionidae); and larvae and nymphs have been collected from Passeriformes (Turdidae) (Guglielmone et al., 2020),

Hyalomma lusitanicum is probably the most abundant exophilic tick in the Central and the Southern part of the Iberian Peninsula (Ruiz-Fons et al., 2006; Basco, 2008; Valcárcel et al., 2015, 2016b). Infestation on ungulates is characterized by a negative binomial distribution typical of most parasitic diseases (Valcárcel et al., 2016b). Study results on infestation prevalence and intensity vary greatly (<41% to > 97%) (Ruiz-Fons et al., 2006; Santos-Silva et al., 2011; Valcárcel et al., 2016b) probably due to climatic and environmental differences, as well as different methodological approaches. It is worth noting that in most ungulate infestation studies, *H. lusitanicum* are collected manually leading to a significant undersampling because the number of hand-collected specimens is much lower than the true tick load, as explained in the section on host sampling.

Table 6.

The behavior of *H. lusitanicum* seems to indicate that it is not very anthropophilic and it should be considered as only a sporadic parasite of humans (Guglielmone et al., 2020; Valcárcel et al., 2020). However, other authors report relatively frequent human infestation in Portugal (Santos-Silva et al., 2011) and reports of attachment in humans have been rising in recent years, the preferred feeding sites being the lower extremities (23.5%), followed by the back and lumbar area (17%), abdomen (13.5%), head and neck (11.5%), thorax (10%), groin and genitals (9.5%), arm and deltoid (6.5%), armpit (6.0%), navel (2.0%) and buttock (0.5%), especially in males over 14 years of age (Bellido-Blasco et al., 2021).

Seasonality

The seasonal pattern of host-seeking *H. lusitanicum* adults is well established in meso-Mediterranean areas. Adults start questing/hunting around March, the number rising quickly and reaching its peak in May–June and then diminishing until the end of the year, with a second smaller peak in September–October. This pattern was repeated in the three forest types studied -eucalyptus, evergreen oak and olive forests- although tick abundance was higher in eucalyptus forest as it offers a more suitable refuge for hosts and ticks (Valcárcel et al., 2016a). This is probably due to the strong influence of host behavior and habitat preference as has been observed in other tick species (Ginsberg et al., 2002; Dobson et al., 2011).

Regarding host infestation patterns, up until the end of the 20th century *H. lusitanicum* adults infested ungulates mainly from April to November in meso-Mediterranean (Habela et al., 2000). Today, however, *H. lusitanicum* can be found on hosts the year round, although the activity of immature specimens is more limited. Larvae are present from April to September (peak in May–June) and nymphs start to emerge approximately one month later, from May to November (peak in June–July), especially in wild rabbits but also in red deer (Sánchez et al., 2022; Valcárcel et al., 2016b). Adults feed on ungulates during the entire year, starting with small numbers, gradually increasing during the spring and peaking between May and July. They then progressively decrease to their lowest numbers in December, but with a small peak in September-October. It is important to note that the two peaks of host seeking adults coincide with the calving and rutting seasons of red deer, when vegetation provides shelter for hosts and ticks. Immature ticks contribute to the highest peak of red deer *H. lusitanicum* infestation, in spring, while engorged females may be collected during the whole year (Valcárcel et al., 2016b, 2020). Different tick stages overlap several times per year (Valcárcel et al., 2016 a,b, 2020). There is a balanced male/female ratio of host seeking *H. lusitanicum* throughout the year. However, the sex ratio is unbalanced on hosts because females leave the host quickly after engorgement while males remain for longer periods. Therefore, the number of males collected from red deer is usually higher than that of females, especially during cold months.

***Hyalomma lusitanicum* and health problems**

Mammal Meat Allergy

As well as other species of ticks, *H. lusitanicum* has been recently related with Mammal Meat Allergy (MMA) (Briones et al., 2022, 2020). In the mid-2000s, cases of delayed anaphylaxis were first detected in the USA after ingestion of red meat or intravenous infusion of the drug cetuximab (Steinke et al., 2015). These cases were very puzzling because the affected patients had never before suffered from this hypersensitivity. Patients were found to be generating high levels of IgE against an epitope, α-Gal, present in both the meat and the drug. The link between tick bites, specifically *Ixodes holocyclus* Neumann, and MMA was first described in 2007 (Van Nunen et al., 2009). It was subsequently confirmed that the α-Gal epitope was the trigger for the allergic manifestations of MMA, as well as its association with tick bites, in this case *Amblyomma americanum* L. (Commins et al., 2011). Some authors suggest that the risk factors for developing MMA are the level of exposure to tick bites (risk professions and activities) and the absence of blood type B (Cabezas-Cruz et al., 2017). Cases of MMA have been detected in the Iberian Peninsula but the presence of α-Gal in native ticks has only recently been detected, especially in salivary glands and in the digestive tract of fed and unfed *H. lusitanicum and R. bursa* with higher concentrations in *H. lusitanicum* (Briones et al., 2020, 2022). Thus, it can be assumed that some tick species are able to produce the epitope and pass it on during feeding. The α-Gal joined proteins in the salivary glands of *R. bursa* and *H. lusitanicum* ticks have a molecular weight of approximately 250 kDa and 37 kDa (Briones et al., 2020, 2022).

Hyalomma lusitanicum can harbor several microorganisms that are harmful to humans and other animals (Table 7). It is a well-known vector and reservoir of Crimean Congo Hemorrhagic virus (CCHFv). Since 2010, different CCHF viral strains have been detected in Spain in adult *H. lusitanicum* feeding on animals, particularly on red deer and in host seeking adults of this species (Estrada-Peña et al., 2012; Cajimat et al., 2017; Negredo et al., 2019; Moraga-Fernández et al., 2020; Sánchez-Seco et al., 2021). This virus appears to be widespread in Spain where it may have been introduced by migratory birds and trade in live animals harboring infected ticks (Portillo et al., 2021). Fortunately, human CCHF cases are still rare in Western Europe where the virus also has been detected in other tick species (*H. marginatum, D. marginatus, R. annulatus* and *I. ricinus*) (MoragaFernández et al., 2020; Sánchez-Seco et al., 2021).

Regarding other viruses, recent studies have discovered *H. lusitanicum* infected with Myxoma virus, a pathogen found in wild lagomorphs (García-Pereira et al., 2021), and with the hepatitis E virus (HEV) (Rivero-Juarez et al., 2021). However, the role of *H. lusitanicum* in the transmission cycle of these two viruses is still unknown and warrants further study.

Hyalomma lusitanicum is considered an effective vector of *Theileria annulata* in Spain (Viseras et al., 1999). Transmission of *T. annulata* by *H. lusitanicum* has not yet been documented in Africa, although this could be happening in parts of Morocco and Algeria (Gharbi et al., 2020). *Hyalomma lusitanicum* also has proven to be a vector of *Theileria equi* Mehlhorn, Schein (Zapf and Schein, 1994) and allegedly of other *Babesia/Theileria* spp. (Díaz-Cao et al., 2021) that have been detected in ticks by means of molecular methods. For example, in Spain, *H. lusitanicum* was suggested to be the vector of *Babesia pecorum* sp. nov. a species detected in farmed red deer (Jouglin et al., 2014).

Hyalomma lusitanicum also has been associated with several tick-borne bacteria, namely Rickettsiales (*Anaplasma phagocytophilum* Foggie, Dumler et al.*, Anaplasma platys* Dumler et al., *Ehrlichia* spp., *Rickettsia* spp.), *Coxiella burnetii* Derrick, Philip*, Borrelia burgdorferi* sensu lato Johnson et al. emend. Baranton et al., and *Franscisella tularensis* McCoy and Chapin, Dorofe'ev (Table 7) - with *Francisella*-like endosymbionts (FLE) being its primary symbionts (Díaz-Sánchez et al., 2021). Specifically, transovarial and transstadial transmission of *C. burnetii*, the agent of Q fever, have been demonstrated; however, the true vector competence of *H. lusitanicum* for this bacterium has not yet been confirmed (González et al., 2020; Sánchez et al., 2022). *H. lusitanicum* might also be involved in spotted fever group Rickettsiae transmission (Remesar et al., 2021).

Table 7.

Control

Tick populations are generally part of the natural environment and may play a role in maintaining its equilibrium. However, under certain circumstances, mostly caused by human intervention, ticks do pose a health risk to humans and domestic and wild animals. In these cases, control measures need to be put in place. The battle against ticks dates to ancient times and some of the traditional practices are still in use today, coexisting with novel control methods. In the case of *H. lusitanicum,* all stages are widely distributed in different ecosystems and parasitize a wide range of hosts making eradication impracticable but it is desirable to keep the population as low as possible to reduce infestation levels and the risk of pathogen transmission.

In the environment

The aim of these methods is to modify tick microhabitats by reducing the survival of non-parasitic tick stages. Among traditional tick control practices, the controlled burning of pastures reduces the abundance of ticks that are directly affected by the initial fire, or indirectly by the effect that burning has on vegetation and hosts (White and Gaff, 2018). However, reduction in tick abundance through this method is temporary, returning to previous levels or even higher densities within one or two years (Drew et al., 1985; Stafford et al., 1998; Cully, 1999; Allan, 2009). We did not find any report on this control measure in relation to *H. lusitanicum.*

Another traditionally used measure is environmental modification such as mowing of weeds and grasses, which helps to reduce the soil moisture needed for tick survival and removes leaf litter that serves as a refuge for ixodids (Clymer et al., 1970; Schulze et al., 1995). The effectiveness of this technique depends on the physiological requirements of each species as ticks are also well adapted to dry environments (Uspensky, 2002). However, these practices require regular maintenance (Wilson, 1986). In addition, leaf litter removal may negatively impact other arthropods that depend on such habitats, including many pollinators (Ginsberg et al., 2017). We have observed a large reduction in the host-seeking *H. lusitanicum* abundance following the clearing of *Eucalyptus globulus* Labill (Myrtales, Myrtaceae) or *Mimosa pudica* L. (Fabales, Fabaceae) forests, and also after the plowing of olive groves (unpublished data).

Worker safety and economic considerations, as well as the risk of environmental pollution when treating large tracts of land directly to control ticks, limits the use of chemical control. However, some acaricides can be applied to limited areas such as stables, barns and small pastures (Drummond, 1983). A low-pressure application with a non-persistent acaricide can have a strong immediate suppressive effect, but a very limited impact on the abundance of host-seeking ticks within a few days or weeks after application. In contrast, high-pressure applications with persistent acaricides maximize the likelihood of efficacy (Eisen and Dolan, 2016) but pose a higher risk of detrimental effects on other beneficial arthropods.

There has been limited published research conducting testing the effect of acaricidal active ingredients on *H. lusitanicum* (Selles et al., 2021). Oxalic acid (OA) is one of the most common organic treatments for varroosis in honey bees worldwide and appears to be compatible with organic farming and would be relatively safe for honey bees and other insects. Its efficacy has been demonstrated in laboratory and field trials against host seeking *H. lusitanicum* adults (Olmeda et al., 2008; Valcárcel et al., 2014). These studies have shown high effectiveness in tick control with a concentration to 50% mortality (LC_{50}) of 0.22 mg at 24h and 0.125 mg at 72h in vitro, while in the field results depended on the particularities of the microhabitat and application method and were not necessarily related to product concentration. Efficacy was higher in pine areas than in olive or eucalyptus (tick reduction rates of up to 100%, 80% and 62.5%, respectively) (Valcárcel et al., 2014), probably because the latter offers better refuge for ticks. When 6.3% OA was applied by hose to the ground and vegetation, the reduction of host seeking *H. lusitanicum* adults ranged from 78 to

100%, but a 3% concentration of OA achieved a reasonable 81% tick population reduction and consumed only half the amount of product (Valcárcel et al., 2014). In the same study, application of 10% OA using an ultra-low volume (ULV) system consumed much less product and covered a larger surface area in much less time than with a hose and afforded a similar reduction (76%) of the *H. lusitanicum* tick population. Other factors such as system handiness must be considered as a hose application system can access certain places where the ULV cannot, limiting the use of the ULV to roadways and areas adjacent to them or which are easily accessible by vehicle. Lastly, before considering OA applications, it is important to know that it degrades rapidly in the field and therefore should be applied precisely on the days when tick abundance is the highest, avoiding rainy or humid days to prevent dilution.

On the hosts

Traditionally, host animals have been managed to reduce tick abundance, as the distribution and number of ticks depends to a large extent on the movement and density of hosts (Wilson et al., 1985; Estrada-Peña et al., 2008; Gilbert, 2010b; Kilpatrick et al., 2014). The effectiveness of these measures depends on the type of tick cycle to be controlled and the tick species ability to survive in the environment. For example, rotation of grazing areas is a measure applicable to monotropic species causing ticks to die of starvation in the absence of hosts for a prolonged period (Hernández et al., 2000). In non-monotropic species, action can be taken to reduce the density of the main host or pathogen reservoir (Barandika et al., 2007; Ginsberg, 2014). Population control and restricted access to certain areas are among the most common practices. These measures are often applied to deer, given their importance in maintaining certain tick species in the environment (Gray, 1998). In other cases, barriers have been used to limit deer access to control *I. scapularis*, *A. americanum* and *I. ricinus*, for example, although their effectiveness can only be analyzed over the long term (Stafford, 1993; Ginsberg et al., 2002; Kilpatrick et al., 2014). Limiting factors can also include certain farm types, the existence of secondary hosts and the effect of habitat fragmentation (Ginsberg, 2014). The fact is that in the absence of the main host, another secondary host may maintain ticks for long periods. In a particular meso-Mediterranean area, the exclusion of ungulates for 16 years, the main hosts of *H. lusitanicum* adults, reduced the infestation of wild rabbits by immature *H. lusitanicum* from 79% to 2% (Valcárcel et al., 2017). In this case, the disappearance of *H. lusitanicum* also led to the increase of *Rhipicephalus pusillus* Gil Collado, a tick species with lower vectorial capacity. However, the reduction of a tick species could be negative if it is replaced by a new species with high invasiveness and spread or greater vectorial capacity especially if it becomes a dominant tick species.

Extensive use of chemical control by direct administration of acaricides and tick repellents remains the most effective and practical strategy, despite limitations due to the rapid development of resistance and environmental impact (Kunz and Kemp, 1994). Acaricides can be applied orally or topically by dipping, spraying, collaring, spot-on and pour-on techniques. Choice of the type of administration depends on the ease with which animals can be handled. In the case of wild animals, where management is limited or non-existent, environmentally-adapted, host-specific methods need to be used. It is possible to take advantage of the concentration of animals at feeders and drinkers to apply treatments. An example is acaricide-treated rollers placed on the sides of the hopper that brush against the head, ears and neck, the preferred attachment sites for certain tick species (Pound et al., 2000).

Use of selective feeders allows acaricides to be administered to target host species while avoiding consumption by other non-target host species and thus reduce acaricide costs. This method has been successfully used to control *H. lusitanicum* in the wild (Valcárcel et al., 2015) and has been recommended to better manage other diseases affecting wildlife (Martínez-Guijosa et al., 2021).

Commercial acaricides, such as pyrethrins or macrocyclic lactones, to control ticks infecting wild animals should be carefully administered to reduce the chance of pesticide contamination (animal products, soil, water, vegetation, etc.). More field studies need to be conducted under ecologically-friendly conditions. An example is spinosins, an insecticide-acaricide produced from the fermentation of metabolites of the actinomycete bacterium *Saccharopolyspora spinose* (Pseudonocardiales, Pseudonocardiaceae) (Davey et al., 2001; Mayes et al., 2003). Spinosins have exhibited acaricidal activity against *R. turanicus* and *Argas persicus* Oken (Brito et al., 2011; Cetin et al., 2009) and has also been tested under laboratory and field conditions to control the infection of wild rabbits by immature *H. lusitanicum* with promising results (Valcárcel et al., 2015). According to Valcárcel et al. (2015), a 50% tick reduction was achieved during the peak infestation period, as wild rabbits, which had access to other non-medicated food, accepted the medicated product after 10-15 days despite taste/odor rejection observed in laboratory rabbits.

Aware of growing interest in biopesticides to control pathogens, many laboratories have focused efforts on screening natural products, some with great success (see biopesticides section).

Acaricidal resistance

The multi-host tick species *H. lusitanicum* is very difficult to manage. Today, tick control is based heavily on the application of chemical acaricides worldwide. However, tick resistance to acaricides has hampered this control method. Studies on tick resistance to commonly used chemicals have mostly focused on ticks of the genus *Rhipicephalus*, specifically the aggressive cattle tick *Rhipicephalus microplus* (Adehan et al., 2016; Chevillon et al., 2007; Kumar et al., 2020; Souza Higa and de Oliveira Souza Higa, 2015) while the resistance of *Hyalomma* species, particularly *H. lusitanicum,* is less documented. One-host ticks have a short life cycle and all life stages occur on the same host. The result is high exposure to frequently used acaricides and high resistance selection pressure compared to multi-host ticks (Singh et al., 2014). However, the results of the limited resistance research conducted on multi-host tick species closely related to *H. lusitanicum* are alarming.

A preliminary study reported the possible resistance of *H. lusitanicum* to amitraz. *Hyalomma lusitanicum* larvae and adults were subjected to the immersion test (LIT and AIT) and the contact test, reaching 100% and 82.3% mortality at 24 hours, respectively (González et al., 2019). In a study by Shyma et al. (2012) using the larval packet test (LPT), *H. anatolicum* exhibited tolerance to deltamethrin and cypermethrin at ten research sites (Resistance factor (RF) = 1.79–4.62), and to diazinon at six sites out of a total of twenty study sites, while

H. anatolicum ticks exhibited a higher-grade resistance level II (RF = 5.1–25.0) to deltamethrin at one site. *Hyalomma anatolicum* isolates exhibited resistance to deltamethrin and diazinon in the study conducted by Gaur et al. (2016) using an AIT. In a resistance study conducted by Kumar et al. (2016) using AIT and LPT, three *H. anatolicum* isolates out of 11 tested exhibited level I resistance against deltamethrin (RF = 1.79–2.52), cypermethrin (RF= 2.0-3.95) and diazinon (RF = 1.32–2.18). Moreover, *H. anatolicum* field isolates exhibited variable resistance to amitraz using a modified LPT in a study conducted by Jyoti et al. (2019). Resistance of the multi-host tick species *H. marginatum*, closely related to *H. lusitanicum,* was assessed in a study conducted by Elhachimi et al. (2022), which concluded that the susceptibility of *H. marginatum* to diazinon and amitraz was variable across the nine research sites, suggesting that acaricide resistance is developing.

As researchers are aware of resistance risks, further studies are needed to determine the acaricidal resistance of *H. lusitanicum* to commercial chemicals used in the host and its environment, and more alternatives are needed to mitigate *H. lusitanicum* resistance.

Vaccines

Host immunization against tick infestation is a promising method to control ticks and tick-borne pathogens (Rego et al., 2019). Research has been conducted to detect and characterize the hydrolases, including proteases and esterases, in adult *H. lusitanicum* extracts (Giménez-Pardo and Martínez-Grueiro, 2008) but no information is available on the efficacy of vaccines against *H. lusitanicum*.

Biological control is the effective use of a pest's natural enemies as biological agents. This includes predators, parasitoids and pathogens (virus, bacteria, insects, fungi, etc.) whose use should be considered, where feasible, as an integral part of a successful integrated pest management plan. Only a few tick natural enemies have been evaluated to date as potential tick biocontrol agents (Samish et al., 2004) and very few biological control agents have been tested against *H. lusitanicum* (Selles et al., 2021).

Predators such as certain spiders, ants, rodents, lizards and birds, help reduce tick populations and so their numbers should be preserved within tolerable levels (Samish and Rehacek, 1999). Although anecdotal, the scorpion *Buthus ibericus* Lourenço & Vachon (Scorpiones, Buthidae) was observed feeding on an engorged *Hyalomma* female in an area where *H. lusitanicum* is abundant (Lindström, 2017).

Concerning parasitoids, only the *Ixodiphagus* wasp genus has been described as a biological control agent for ticks. This is a small wasp that deposits its eggs in the body cavity of immature ticks. After hatching, wasp larvae feed on tick tissues and wasps emerge as adults by tearing the cuticle of the tick, which dies in the process (Mwangi et al., 1997). *Ixodiphagus* has been found in *R. bursa* in an area where *H. lusitanicum* is present (Foldvari et al., 1992), but no cases of it parasitizing *H. lusitanicum* have been reported.

Ticks are infected by a wide range of pathogens such as viruses, bacteria, fungi and nematodes. Some soil constituent fungi are entomopathogenic fungi and may be particularly important in tick control. As some entomopathogenic fungi strains develop in living arthropods, they have been commercialized for plant pest control (Fernández et al., 2005). Many laboratory experiments have been conducted to analyze the virulence of various isolates, mainly from *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales, Clavicipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales, Clavicipitaceae) for application against several tick species such as *R. (B.) microplus, R. sanguineus, H. anatolicum anatolicum, Haemaphysalis qinghaiensis* Teng*, Dermacentor variabilis* Say and *I. scapularis* (Zhioua et al., 1997; Gindin et al., 2002; Barci et al., 2009; Fernandes et al., 2011; Sun et al., 2011, 2013; Perinotto et al., 2012; Cafarchia et al., 2015). Other fungal species such as *Purpureocillium lilacinum* (Thom) Luangsa-ard, Hou- braken, Hywel-Jones & Samson (Hypocreales Ophiocordycipitaceae) and *Lecanicillium lecanii* R. Zare & W. Gams (Hypocreales, Cordycipitaceae) prepared in aqueous and oily suspensions, were effective against *Amblyomma cajennense* Fabricius and *R. (B.) microplus* (Angelo et al., 2010; D'Alessandro et al., 2012).

The pathogenicity of entomopathogenic fungi depends not only on the fungal strain, but also on the tick species and its stage of development (Kirkland et al., 2004). However, even when fungal efficacy has been demonstrated under laboratory conditions, the effectiveness of field treatment depends on environmental conditions and application methods. There are reports of the ixodicidal efficacy of entomopathogenic fungi when applied to soil for the control of *I. scapularis* and *Rhipicephalus appendiculatus* Neumann (Kaaya et al., 1996; Benjamin et al., 2002; Hornbostel et al., 2004; Stafford and Allan, 2010). The efficacy of a commercial strain of *B. bassiana* against *H. lusitanicum* in wild rabbit burrows was evaluated using a fog sprayer under field conditions (González et al., 2016a). The treatment significantly reduced the intensity of *H. lusitanicum* infestation in rabbits for at least two months, the best results coming in spring when the parasitic index (PI) fell by 78.63% and 63.28% on day +30 and +60, respectively ($P < 0.05$). PI reduction in summer was more limited, 35.72% on day +30 ($P = 0.05$). The authors suggested that the efficacy of applications inside burrows could be temperature-dependent and that such applications could be a cost-effective alternative to rabbit tick control under field conditions.

Biopesticides

The use of natural products in tick control is not new. Macrocyclic lactones were obtained by fermenting actinomycetes in the late 1970’s and marked a revolution in endo- and ectoparasite control. However, their efficacy in tick control is controversial as results are highly variable (Davey and George, 2002; Perez-Cogollo et al., 2010). More recently, oxalic acid and spinosins have proven to be effective and fast-acting against *H. lusitanicum* (Olmeda et al., 2008; Valcárcel et al., 2014, 2015). Oxalic acid has a short activity and toxicity (TOXNET, 2007) and spinosins have long residual activity and low toxicity (Breslin et al., 2000).

Aware of interest in biopesticides in vector control, many laboratories have focused their efforts on screening natural products, with great success in some cases. There are advantages to using natural products to control ticks, but there are limitations as well. Advantages include the synergistic effect of extracts not found in single compound applications, they offer an alternative in the control of resistant ticks, environmental friendliness and minimal health impact (CDC, 2018). The first limitation is inconsistency in the composition (and hence the efficacy) of certain extracts such as essential oils (EOs). Extract standardization entails first identifying the active compounds. Other limitations could be photosensitivity and high volatility, limiting residual activity depending on the form of presentation (George et al., 2014).

Among plant-derived products, EOs are particularly attractive for effective tick control due to their theoretically low toxicity, low environmental permanence and complex chemistry that hinders the development of resistance. EOs are naturally produced by plants as secondary compounds and can be obtained for commercial use by various forms of distillation. Some of the most used botanical families are Asteraceae, Fabaceae, Lamiaceae, Meliaceae, Solanaceae and Verbenaceae (Benelli et al., 2016). Studies on the ixodicidal effect of natural products have targeted *Amblyomma sculptum,* Berlese*, A. americanum, A. cajennense, Dermacentor nitens* Neumann, *Haemaphysalis bispinosa* Neumann, *Hyalomma aegyptium* L.*, H. marginatum, Hyalomma scupense* Schulze*, H. lusitanicum, I. ricinus, Rhipicephalus (B.) decoloratus* Koch*, R. sanguineus* and, above all, *R. (B) microplus* (revised by Selles et al. (2021)). Many EOs and their main compounds recently have been tested against *H. lusitanicum* larvae with promising efficacies, but they need further development (Table 8). A 5 µg/µl concentration solution (50 µl) of eremophilane sesquiterpenes from *Senecio adenotrichius* de Candolle (Asterales, Asteraceae) was deposited over 25 mg of cellulose allowing solvent evaporation and, then tested against *H. lusitanicum* larvae obtaining 88% larval mortality by LPT (Patent number: P201330081, under the title “*Uso de sesquiterpenos eremofilanos como garrapaticidas*”).

Table 8.

It is now recognized that tick populations cannot be controlled, let alone eliminated, by simply applying an acaricide treatment during the highest peak of animal infestation by ticks. The pressure exerted by continuous and poorly designed treatments has led to the current situation of resistant tick populations with negative environmental consequences. The current tendency is to combine specific strategies at different points in the tick cycle to reduce pest numbers without elimination, i.e. keeping the population within a tolerable limit with minimal environmental impact (Stafford et al., 2017). Tick population control must be tailored for each species taking its phenology, climatic conditions, type of management, host availability, etc. into account. There is no one best stand-alone strategy and an Integrated Pest and Pollinator Management (IPPM) plan, which includes non-pesticidal pest management practices and the replacement of commercial pesticides, needs to be developed [231].

Objectives of an IPPM strategy include appropriate guidelines to prevent infestation, reducing the number of treatments, and establishing critical application times. That is why some of these programs include the application of acaricides (albeit increasingly less frequent due to potential side effects and environmental impacts) combined with other sustainable measures, which achieve better results than when used alone. For example, to control *A. americanum* in the United States, combinations of acaricide applications and vegetation and animal management have been carried out with better results than one single technique (Eisen and Dolan, 2016). Another example is the combination of entomopathogenic fungi with acaricides known to be effective, or with natural products to enhance their efficacy (Hornbostel et al., 2005; Sousa et al., 2011; Webster et al., 2015).

Strategies also have been developed to improve the application of enthomopathogenic fungi. For example, by impregnating nesting material used by wild mice with the fungus (Hornbostel et al., 2005), or by attracting ticks with pheromones to fungus-treated areas (Maranga et al., 2006). As described above, *H. lusitanicum* can infest a wide range of different domestic and wild hosts. Integrated control seems to be the best option to reduce tick infestations of domestic ungulates, i.e. application of several products during the course of the year (organophosphates, macrocyclic lactones, fluazuron, entomopathogenic fungi and vaccines), thus increasing the performance of animals as the number of ticks decreases (Rodríguez-Vivas et al., 2014). Wildlife management is more difficult than domestic livestock management, but, taking *H. lusitanicum* patterns into account (host-seeking adults, infestation of wild rabbits and red deer), together with field studies and the combined application of several control measures throughout the year (Cota Guajardo, 2015; Valcárcel et al., 2019) will presumably result in a considerable reduction of the tick population in subsequent years (Figure 2).

Conclusions:

The purpose of this study was to furnish the latest available information on *Hyalomma lusitanicum* with a view to controlling it in areas where it is currently found and also in new areas where this hard tick species could establish itself in the near future. This tick species may play an emerging role in maintaining the agents of important zoonoses including Crimean-Congo hemorrhagic fever virus and Q fever. However, vectorial capacity/competence for CCHFv and the true vector competence for *C. burnetii* has not yet been confirmed. Knowledge about this tick species is limited to its distribution area and is not always easily accessible. Therefore, gathering comprehensive information on *H. lusitanicum* will help pest surveillance officers to better understand its adapted strategies and, above all, will provide them with the necessary tools to control *H. lusitanicum* both in the geographical areas where this tick is endemic and in those where it could become established in the future.

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Author contributions:

(1) The conception and design of the study, F.V.

(2) Acquisition and interpretation of data, F.V., L. E., M. V., L.T., SMA. S., M. K.

(2) Drafting the manuscript or revising it critically, all authors.

(3) Final approval of the version to be submitted, all authors.

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Table 1. Taxonomy of *Hyalomma lusitanicum,* based on Schoch et al. (2020)

Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Acari
Superorder	Parasitiformes
Order	Ixodida
Superfamily	Ixodoidea
Family	Nuttalliellidae
	Argasidae
	Ixodidae
Subfamily	Amblyomminae
	Haemaphysalinae
	Rhipicephalinae
	Hyalomminae
Genus	*Hyalomma*
Species	*Hyalomma lusitanicum*
	Other 26 species

Table 2. Comparison of the length and width (in µm) of the scutum/conscutum of *Hyalomma lusitanicum* compared with other *Hyalomma* spp reported in the literature. In the list we do not consider additional changes in the species denominations and we maintain the names as reported in the respective references: 1 Apanaskevich et al., 2008; 2 Apanaskevich et al., 2008; 3 Apanaskevich and Horak, 2008b; 4 Apanaskevich et al., 2010; 5 Apanaskevich and Horak, 2007; 6 Apanaskevich and Horak, 2006; 7 Apanaskevich and Horak, 2009; 8 Apanaskevich and Horak, 2008a; 9 Torres de Almeida; 10 Tendeiro, 1955.

	Male			Female			
	Length	Width	Length/Width	Length	Width	Length/Width	Ref.
H. albiparmatum	3,890	2,520	1.54	2,160	2,080	1.04	8
H. dromedarii	4,980	3,380	1.48	2,290	2,200	1.04	3
H. franchinii	4,170	2,770	1.5	2,520	2,330	1.08	1
H. glabrum	4,720	3,270	1.44	2,650	2,690	0.98	6
H. impeltatum	3,840	2,400	1.6	2,250	2,170	1.04	7
H. impressum	3,830	2,340	1.64	2,200	2,190	1.01	5
H. isaaci	3,990	2,590	1.54	2,260	2,190	1.04	2
H. marginatum	4,460	2,870	1.55	2,460	2,360	1.04	2
H. nitidum	3,900	2,430	1.6	2,060	2,070	1.00	8
H. rufipes	5,000	3,330	1.5	2,670	2,630	1.02	2
H. schulzei	3,760	6,230	1.66	2,740	2,570	1.06	3
H. scupense	4,140	2,180	1.61	2,190	2,080	1.05	4
H. somalicum	4,270	2,940	1.45	2,340	2,420	0.97	7
H. truncatum	4,090	2,640	1.55	2,160	2,090	1.04	8
H. turanicum	4,270	2,720	1.57	2,470	2,410	1.02	2
H. lusitanicum	3,826	2,492	1.54	2,034	2,013	1.01	UD
	3,660	2,410	1.52	2,040	2,000	1.02	1
	3,486	2,206	1.58	NP	NP	NP	9
	3,588	2,348	1.53	NP	NP	NP	9
	3,650	2,600	1.40	NP	NP	NP	10

UD (unpublished data), NP (not provided).

Table 3. Morphology and measurements (in µm) of larvae of *Hyalomma lusitanicum*.

	(11)	(Apanaskevich et al., 2008)	(12)
Basis capituli	L 127	W 118-140	
Palpi		L 92-106 W 35-40 L/W 2.40-2.83	
Hypostome	L 64 W 34	L 82-102 W 25-30 L/W 2.83-3.78	
Genua I		L 102-126 W 46-50 L/W 2.20-2.56	
Basis capituli	Marked lateral basidorsal cornuas without posterior basidorsal cornuas and slope of the tectum is domed Dorsal view: hexagonal. Wider than long Ventral view: Solid, rounded at rear edge, no ridge or spur, 1 pair of subhypostomal (or posthipostomal) setae	Ventral view: subrectangular Dorsal view: subhexagonal Apexes of dorsolateral projections are directed slightly forward and they are acute and distinct in ventral view	Dorsal view: trapezoidal with marked lateral expansions Ventral view: rounded posterior edge
Palpi	Segments II and III have bearded setae. Articles II and III welded.		Elongated
Hypostome	Denticulated area have a hypostomal formula 2/2 of 6 to 7 tooth rows	5 large denticles, ½ of hypostome length with abrupt transition to non-denticulated zone	With six teeth of 2/2 rows
Scutum	Significantly wider than long 3 pairs of very short setae At least 1 pair of auriform sensillae near its anterior margin 1 pair of large eyes situated at their widest zone Rounded side angles Posterior margin sinuous	Posterior margin wide rounded Posterolateral depressions indistinct or absent Posterior portion to eyes nearly equal to ½ of scutal length	Hexagonal, wider than long. Eyes placed at the level of highest width
Alloscutum	Dorsal view 2 pairs of mid-dorsal setae, 8 pairs of marginodorsal setae, numerous sensilla, 1 pair is particularly large near the 5th pair of marginodorsal setae (sensilla sagittiforme) Ventral view 3 pairs of sternal setae, 2 pairs of pre-anal setae, 1 pair of anal setae, 4 pairs of premarginal setae, 5 pairs of marginal setae		
Coxa I	2 setae with a less conspicuous flattened ridge-shaped posterior spine	Large subtriangular spur broadly rounded at the apex	
Coxa II	2 setae with a distinctly conspicuous flattened ridge-like posterior spine	Large arched spur	Ridge-like spur
Coxa III	2 setae with a rather conspicuous flattened, ridge-shaped posterior spine	Large triangular spur	Ridge-like spur
Tarso I	Thin with an apical astragalus		

L= length, W= width

Table 4. Morphology and measurements (in µm) of nymphs of *Hyalomma lusitanicum*.

	Perez-Eid and Cabrita, 2003	Apanaskevich et al., 2008	Pérez-Eid, 2007
Total length	1,455		
Scutum	L 455 W 545	L 536-600 W 608-736 L/W 0.78-0.93 Distance from the rear margin of the eye to the end of the shield: 224-264	
Basis capituli	L 317	L 328-384 W 292-320 L/W 1.11-1.22	
Hypostome	L 182 W 91	L 204-250 W 56-66 L/W 3.37-4.21	
Pedipalps	Thinner than in the larva, with separate articles II and III Setae are proportionately shorter than in the larva, a large sensilla on the lower 1/3 of article II	Segment II is proximally narrow and gradually expanding distally	Elongated, in particular articles II
Hypostome	Hypostomal formula 2/2 of about 10 rows of teeth	Denticulate portion with 6-7 large denticles is lesser than twice the length of the non-denticulated zone with abrupt transition between zones	Widened towards its apex and bearing nine-ten teeth in rows 2/2
Capitulum basis	Dorsal view: triangular, wider than high, very angular lateral basal-dorsal horns with a pair of setae inserted near, slope of the tectum is much more gently domed than in the larva Ventral view: massive, very rounded on the posterior margin, has no ridge or horn, but 1 pair of lateral setae		Dorsal view: Triangular with very sharp lateral expansions Ventral View: rounded posterior edge
Scutum	Pentagonal with anterior base, scarcely wider than long, rounded lateral angles slight notch in posterior outline near its lateral angle where well-marked eyes are located 7 pairs of very short setae: 1 pair apical on either side of the cervical furrows, 4 pairs of centromedian, and 2 pairs of posterolateral	Posterior margin is narrowly rounded Slight posterolateral depressions in each side of scutal extremity	Hexagonal, broad with the eyes placed at the level of greatest width
Alloscutum	Dorsal view: 4 pairs of centro-dorsal setae (in the vicinity there are a pair of organs probably precursors of the dorsal foveae of the future adult), 8 pairs of margino-dorsal setae, 8 pairs of laterals setae Ventral view: numerous sternal setae: 3 pairs preanals, 2 postanals, 2 anals, and 6 marginals	Setae narrowing to denticulate apex	
Coxae	3 setae and no spines	Coxal pore present	Without spurs
Coxa I		Long, very broad, U-shaped spurs, median spur shorter than lateral	
Coxa II to IV		Moderate spurs that markedly decrease in size from coxae II to IV	
Spiracle areas		Oval, dorsal prolongation indistinct, short and broad. Submarginl row of perforations incomplete	

L= length, W= width

Table 5a. Morphology and measurements (in µm) of males of *Hyalomma lusitanicum.*

	Walker et al., 2003; Estrada-Peña et al., 2004, 2014	Apanaskevich et al., 2008	Hoogstraal and Kaiser, 1959	Feldman-Muhsam, 1962	Estrada-Peña et al., 2017	Torres Almeida*	Tendeiro, 1955
Gnathosoma							Long capitulum with rounded lateral edges and complely or partially covered with an ivory-colored surface like the dorsal shield
Mouthparts						Long rostrum	
Basis capituli		No lateral projections Dorsal posterior margin is slightly concave	May have dirty white marbling			Rectangular without cornua evident	Rectangular. covered with an ivory-colored surface like the dorsal shield
Palps	The first segment of the palps has more than 5 ventro-median setae	Segment I with more than 5 ventromedian setae	Dorsal surface: reddish to dark brown, extensively marbled when dry				Long and strong Article II larger than article III
Hypostome		Club-shaped, denticle portion is slightly longer than denticle-free portion					Long, claviform, armed with apical corona and with 3/3 dentition (8 to 10 teeth per row)
Spiracle areas	183.06 µm^2 [118.11- 369.70] (UD)						
Dorsal prolongation	Sparse setae	Distinct long dorsal prolongation from body of plate Circumspiracular setae sparse	Thick and long	Tail is quite wide		Long tail Not hirsute circumspiracular area	Very compressed
Perforated portion		Gently curved throughout its length					
Idiosome							Oval, tapering towards the front, without bump at spiracle level
Conscutum	Dark coloured (but may have faint white enamel ornamentation on the scapulae)	Red-brown and pale marbling clearly visible wide and oval-shaped L 3,010-4,080 W 1,990-2,720 L/W 1.40-1.68	Reddish-brown L 3,250-4,050 W 2,400-2,800	Brown or red-brown with a characteristic white-yellowish colour at the anterior part down to the level of the eyes which sometimes extends laterally and may almost reach the posterior end of the scutum. In rare cases, covers the whole scutum	Relatively dense large, medium and short punctations	Presence of eyes Dark brownish honey colour Whitout enamel cover in scapulae and median field areas Usually strong and evident depression of the posterior part of ther scutum, between the	Normal-brown to dark-brown, coated with ivory enamel, interrupted at punctations level Convex and more or less prominent at the front Heavily depressed in the posterior zone

				and occasionally also the basis capituli L 3,000-4,000 W 2,000-2,800		elevations formed by the fusion of the festoons one to three L 2,540-4,318 W 1,525-2,794	and forming a pronounced hump in the transition zone Slight strangulation at the level of the spiracles L 2,400-2,800 W 3,250-4,050
Punctations	Large but there are also small and very large, the largest are located in marginal areas and caudal fields	Medium and small punctations markings dispersed over the conscutum, denser on the caudal area and less dense on elevations demarcating this part	Few large and scattered fine punctations in central field that become more numerous and very marked laterally and in cervical and scapular areas	The whole scutum, apart from the central part, is quite dotted with medium and large dots punctations are especially dense in the caudal area		Large and medium size punctations mainly focused near by the scapulae and anterior median field, between the cervical grooves Sometimes densely punctuated	Large, more or less numerous, clustered particularly on the scapulae and lateral zones and dispersed in the central zone and in festoons zone
Lateral grooves	Short	Short, < 1/3 of the conscutum L and superficial or absent	Very short or absent		Superficial	Absence or superficial and hardly detectable almost reaching the leg IV	Very short, not extending towards the front of the projecting portion corresponding to the three external festoons
Cervical grooves		Short, < 1/3 of the conscutum L and superficial or absent	Originate as deep pits and extend as smooth grooves to the ½ of scutum length Dirty white marbling between cervical grooves	Deep, broad and reach beyond eye level			Deep and divergent, extending beyond the eyes
Scapular areas			Dirty white marbling				
Marginal grooves		Short furrow-like up to ¼ of conscutum length		Absent or low marked			
Posteromedian grooves	Present	Separated from parma by wart-like patch	Faint and may reach or not the median festoon	Only median groove is generally present		Deep at rear, reaching or not the central festoon limiting grooves, and anteriorly superficial, almost gaining the fovea proximity	
Paramedian grooves	Absent	Well defined	Short and indistinct More or less obscured by punctations and sometimes rugosity of caudal depression			Obsolete, although in one quite flattened specimen they were pretty evident. Dark brownish honey colour or pale, light brownish honey colour.	Obliterated

Posterior ridges	Two		Dirty white marbling				
Central festoon	Pale but may be dark	Present or absent with no sclerotization or only anterior half is sclerotized	Absent or faintly present	Generally pigmented but sometimes it is wholly or partly unpigmented		Absence of parma Absence of bridge over Same dark brownish honey colour of scutum Some show a partially pale colour	With the same degree of chitinization as the rest of the dorsal shield and never forming a true unchitinized parma
Paracentral festoons	Separated anteriorly	4 distinct festoons	4 distinct festoons Laterally festoons are fused and continuous with the raised ridge bodering the caudal depresion	Pigmented		Fusion of the festoons one to three, in some individuals are less evident	Fused into a single protuberance, laterally framing the depressed caudal field
Cervical fields	Apparent						
Caudal depression	Present	Well defined laterally demarcated by discreet ridges		Flanked by elevated ridges		Strong and evident formed by the fusion of the festoons one to three densely punctate with small sized punctations, being possible to find some few and scattered large punctations Sometimes it is not so evident	
Legs	White enamel ornamentation as irregular patterns or marbling on dorsal and lateral surfaces, ventral surface without white enamel	Ivory-coloured enamelling diffuse on dorsal and lateral surfaces of each segment of the legs; ivory coloured bands indistinct	Reddish-brown, they are usually very marked and extensively marbled when dry	Marbled appearance with a light-coloured spatter		Less dark brownish honey than the scutum, with a spotted pale honey enamel pattern	Progresively stronger from the 1st to the 4th pair Reddish-brown and ivory coloured enamelling like in the capitulum and in the dorsal shield With imprecise contour and with brown speckles
Coxa I		Posteromedian and posterolateral spurs long, subequal in length or posterolateral spur longer than posteromedian spur, close together, tapering to apices			Posterolateral spur longer or subequal to posteromedian spur	Deeply divided into a narrow external branch and a wider internal branch	Divided into two sharp branches, the inner one clearly stronger and equipped with a more or less accentuated protrusion
Coxa II		Posterolateral spur Distinct, broadly arcuate					Broad inner crest and a blunt outer spur

		posteromedian spur poorly developed, very broadly arcuate					
Coxa III		Posterolateral spur distinct, broadly arcuate posteromedian spur poorly developed, very broadly arcuate					Broad inner crest and a blunt outer spur
Coxa IV		Posterolateral spur distinct, broadly arcuate internal spur distinct, triangular					Broad inner crest and a blunt outer spur
VENTRAL VIEW Subanal plates	Distinct and aligned with adanal plates	Large and rounded	Large, positioned directly at the rear of them usually rounded at the back	Anal plates have a fairly long median transverse prolongation		In line with the central axis of the adanal shields, even in the engorged individual	Small, located exactly behind the adanal plates on a non-chitinous hemispherical base, sightly divergent in engorged specimens
Adanal plates	Shape with square ends	Long and wide; lateral margin slightly convex, antero-median margin concave, median projection large, posteromedian margin straight and posterior margin slightly concave	Robust, rather short, rectangular, and mostly indented on the mid-posterior margin	Fairly long median transverse prolongation	Broad, posterior margin is concave	Extending themselves in a narrow and smooth tip behind the anus Tegument from dark brownish honey colour, undistinguishable from scutum, to pale brownish honey colour	With a quadrangular posterior part, the outer edge convexiline or sub-rectilinear in its posterior 2/3 and rounded at the front The inner edge is straight and project an inner point behind the anus
Accessory adanal plates		Narrow and trapezoidal with well-defined lateral and posterior end border				Present	Well chitinized, with a sharp posterior-internal angle and a more or less rounded posterior-external angle
Genital aperture						At front level of the coxa II	

UD (unpublished data); * there is no date of the publication

Table 5b. Morphology and measurements (in µm) of females of *Hyalomma lusitanicum.*

	Walker et al., 2003; Estrada-Peña et al., 2004, 2014	Apanaskevich et al., 2008	Hoogstraal and Kaiser, 1959	Feldman-Muhsam, 1962	Estrada-Peña et al., 2017	Torres Almeida*	Tendeiro, 1955
Gnathosoma							Long capitulum with rounded lateral edges and complely or partially covered with an ivory-colored surface like the dorsal shield
Mouthparts						Long rostrum	
Basis capituli		Short dorsal lateral projections that are not visible from a ventral view Dorsal posterior margin is slightly concave	Reddish to dark brown, extensively marbled when dry	White-yellowish colour less distinct		Rectangular without cornua evident	Hexagonal. Covered with an ivory-colored surface like the dorsal shield
Palps	The first segment of the palps has more than 5 ventro-median setae	Segment I with more than 5 ventromedian setae	Dorsal surface: reddish to dark brown, extensively marbled when dry	White-yellowish colour less distinct			Longer than male, particulary article II
Hypostome		Club-shaped, denticle portion is slightly longer than denticle-free portion					Long, claviform, armed with apical corona and with 3/3 dentition (8 to 10 teeth per row)
Spiracle areas	152.23 μm^2 [68.85-360.90] (UD)						
Dorsal prolongation	Sparse setae	Distinct long dorsal prolongation from body of plate Circumspiracular setae sparse	Thick and long	Tail longer than in other *Hyalomma* spp.		Long tail Not hirsute circumspiracular area	Very compressed
Perforated portion		Curved and relatively narrow					
Idiosome							Yellowish-brown to dark-brown, ovoid and slightly narrow at the front
Scutum	Dark coloured, but it has faint white enamel ornamentation on the scapulae Posterior margin is sinuous with distinctly concave outlines behind the eyes	Red-brown, pale marbling length is similar to width with prominent posterolateral angles L 1,650-2,410 W 1,680-2,340 L/W 0.94-1.12	Reddish to dark brown, extensively marbled when dry L 2,200 W 2,000	As long as wide Brown or red-brown with a characteristic white-yellowish colour Rather dotted with medium-sized dots and medium-sized and large dots, usually more or less cardiac in shape L 2,000 W 2,000	Marbeled white spots that converge irregularly and extend to the basis capituli, palpi and legs		More or less dark-brown and covered by an ivory-enamel similar to the male but also thicker, particularly dense in the middle zone L 1.95-2.00 W 1.90-2.05

Punctations	Basically large, located in the cervical and central areas, but they can be small or larger and shallow often joined together and feature a rough internal surface	Punctations are large or medium-sized, dense and evenly distributed	Few large and scattered fine punctations in central field that become more numerous and very marked laterally and in cervical and scapular areas		Relatively dense large and medium punctations		Large and medium agglomerations in the scapular angles and in lateral zones
Scapular grooves	Steep and extend to the posterior scutum margin and are obscured by long line punctations						
Cervical grooves		Moderately deep, extending to posterior margin of scutum	Deep, broad, extending to the posterolateral margin of the scutum, usually with some large punctations and lateral rugosity	Reach the posterior edge			Deep Continuing to the posterolateral edges by cervical depressions, bordered externally by a fringed zone and with coarse punctuations
Marginal grooves							Well creased, encompassing just two festoons
Median groove							Joining behind the limit of the median festoon and almost reaching the level of the fovea
Paramedian groove							Slightly extending beyond the median groove
Cervical fields			Deep	Pits between the cervical and lateral grooves corrugated with very large rough spots			
Lateral grooves		Moderately deep, extending to posterior margin of scutum					
Central festoon							Triangular narrower than the paracentral festoons
Paracentral festoons							Quadrangular, as wide as long
VENTRAL VIEW Genital aperture anterior groove	Shallow and preatrial fold is convex with posterior lips which have a broad V-shape	U-shaped with strongly bulging vestibular portion of the vagina	Genital flap, more or less broadly shield-like in outline, does not bulge in profile, gradually depressed posteriorly	Operculum is shaped like a longitudinally elongated knob which does not cover the vaginal orifice flaps long and wide	Genital opening with convex anterior margin and lateral margins converging to a point with their outline neither prominent nor depressed		Opposite to the anterior part of the coxae II, characterized by the ogival-shaped cleft but more or less open depending on the state of repletion

Porose areas							Separated by a median ridge that divides into two branches, extending along the posterior border of the base of the capitulum
Legs	White enamel ornamentation as irregular patterns on dorsal and lateral surfaces	Ivory-coloured enamelling diffuse on dorsal and lateral surfaces of each segment of the legs; ivory coloured bands indistinct	Reddish-brown, they are usually very marked and extensively marbled when dry	Marbled appearance with a light-coloured spatter			Progresively stronger from the 1st to the 4th pair (less strong than males) Reddish-brown and ivory coloured enamelling like in the capitulum and in the dorsal shield With imprecise contour and with brown speckles
Coxa I		Posteromedian and posterolateral spurs long, subequal in length or posterolateral spur longer than posteromedian spur, close together, tapering to apices			Deeply incised with two contiguous, unequal spurs		
Coxa II and III		Posterolateral spur distinct, broadly triangular with rounded apex, posteromedian spur poorly developed broadly arcuate					
Coxa IV		Posterolateral spur distinct, broadly triangular with rounded apex					

UD (unpublished data); * there is no date of the publication

Table 6. Global parasitic indicator (PI= number of *Hyalomma lusitanicum* per animal) collected on red deer using two different collecting methodologies: manual method and hanging method (hang the animals - or their skins - upside down over a container filled with water for 24/48 h at room temperature to allow the ticks to detach from the host and fall into the water and then filter the water).

Collection method	Manual	Hanging
Red deer (n)	39	36
Ticks collected	2,487	22,337
Mean PI	63.77	620.47

Table 7. Previously published reports of the infection of *Hyalomma lusitanicum* by tick-borne pathogens.

Pathogen	**Country**	**Pathogen characterization**	**Source of *H. lusitanicum***	**Reference**
Anaplasma spp.	Spain	*A. phagocytophilum*	Questing ticks and ticks feeding on animals	Toledo et al., 2009
		A. phagocytophilum	Ticks feeding on wildlife	Díaz-Cao et al., 2021
	Italy (Sicily)		Questing ticks and ticks feeding on domestic animals	Torina et al., 2010
	Italy (Sardinia)	*A. phagocytophilum* and *A.platys*	Ticks feeding on hedgehog	Chisu et al., 2018b
	Portugal	*A. platys*	Questing ticks and ticks feeding on wildlife	Santos-Silva et al., 2017
Borrelia burgdorferi s.l.	Portugal		Ticks feeding on red deer	Santos-Silva et al., 2008
		B. lusitaniae	Questing ticks	Milhano et al., 2010
	Spain	*B. burgdorferi s.s.* and *B. garinii*	Questing ticks	Toledo et al., 2009
		B.afzelii	Ticks feeding on wildlife	Díaz-Cao et al., 2021
Coxiella burnetii	Portugal		Questing ticks and ticks feeding on wildlife	Santos-Silva et al., 2017
	Spain (Canary Islands)		Ticks feeding on animals	Bolaños-Rivero et al., 2017
	Spain		Ticks feeding on wild rabbits and red deer	González et al., 2020, 2019; Sánchez et al., 2022
			Questing ticks and ticks feeding on animals	Toledo et al., 2009b
Ehrlichia spp.	Italy (Sicily)		Ticks feeding on domestic animals	Torina et al., 2010
Francisella tularensis	Spain		Questing ticks	Toledo et al., 2009
Francisella-like endosymbionts	Italy (Sardinia)		Ticks feeding on sheep	Chisu et al., 2019
	Spain		Ticks feeding on red deer	Díaz-Sánchez et al., 2021
Rickettsia spp.	Portugal	*R. helvetica*	Ticks feeding on wild boar	Pereira, 2018
	Italy (Sicily)	*R. aeschlimannii*	Ticks feeding on human	Blanda et al., 2017
	Italy (Sardinia)	*R. aeschlimannii*	Questing ticks	Chisu et al., 2018a
	Spain	*R. slovaca* and *Rickettsia sp.*	Ticks feeding on wild boar	Castillo-Contreras et al., 2021
		R. massiliae, R. sibirica subsp. *mongolitimonae, R. slovaca, R. aeschlimannii* and *R. africae,*	Ticks feeding on sylvatic lagomorphs	Remesar et al., 2021
Theileria and *Babesia* spp.	Italy (Sicily)	*T. annulata* and *T. buffeli*	Ticks feeding on cattle	Georges et al., 2001
		Theileria/Babesia spp.	Questing ticks and ticks feeding on domestic animals	Torina et al., 2010
	Spain	*Theileria/Babesia spp.*	Ticks feeding on wildlife	Díaz-Cao et al., 2021
		T. annulata	Field experiment for vectorial campetence	Viseras et al., 1999
Crimean–Congo haemorrhagic fever virus (CCHFV)	Spain		Ticks feeding on red deer	Estrada-Peña et al., 2012
			Ticks feeding on red deer	Cajimat et al., 2017
			Ticks feeding on red deer and cattle	Negredo et al., 2019
			Ticks feeding on red deer and wild boar	Moraga-Fernández et al., 2020
			Ticks feeding on red deer, fallow deer, mouflon, wild boar	Sánchez-Seco et al., 2021
Hepatitis E virus (HEV)	Spain		Ticks feeding on wild boar	Rivero-Juarez et al., 2021
Myxoma Virus	Spain		Ticks feeding on wild lagomorphs	García-Pereira et al., 2021

Hedgehog= *European hedgehog* L. (Eulipotyphla, Erinaceidae), red deer= *Cervus elaphus* L. (Artiodactyla, Cervidae), wild rabbits= *Oryctolagus cuniculus* L. (Lagomorpha, Leporidae), sheep= *Ovis aries* L. (Artiodactyla, Bovidae), wild boar= *Sus scrofa* L. (Artiodactyla, Suidae), cattle= *Bos taurus* L. (Artiodactyla, Bovidae), fallow deer= *Cervus dama* L. (Artiodactyla, Cervidae), mouflon= *Ovis aries musimon* Pallas (Artiodactyla, Bovidae)

Table 8. Essential oils and their main compounds recently tested against *Hyalomma lusitanicum* larvae.

Compound	**Origin**	**Reference**
Camphor 5-methylen-2, 3, 4, 4-tetramethylcyclopenten-2-one	*Lavandula stoechas Lam.**	Julio et al., 2017
Eremophilane sesquiterpenes 1, 3, 4, 8	*Senecio adenotrichius*	Ruiz-Vásquez et al., 2017
β-elemenone germacrone Linalool Thymol	*Geranium macrorrhizum* L.	Navarro-Rocha et al., 2018
Essential oils	*Artemisia dracunculus L.* *Artemisia herba-alba* Asso *Hyssopus officinalis* L. *Lavandula angustifolia* Mill. *Mentha piperita* L. *Mentha suaveolens* Ehrh. *Mentha spicata L.* *Origanum vulgare subsp.virens L.* *Rosmarinus officinalis L.* *Satureja montana* L. *Tanacetum vulgare* L. *Thymus mastichina* L. *Thymus vulgaris* L. *Thymus zygis* Loefl. ex L.	revised by Valcárcel et al. 2021
Industrial essential oils	*Hyssopus officinalis* L. *Lavandula intermedia Super* Emeric ex Loisel. *Santolina chamaecyparissus* L.	Ortiz de Elguea-Culebras et al., 2018
Essential oils with supercritical CO_2, rich in phytol, carvacrol, and hexahydrofarnesyl acetone	*Jatropha curcas* L.	Soto-Armenta et al., 2020

* published as *Lavandula luisieri* in the reference