

# Ixodid ticks on wild donkeys in a Mediterranean nature reserve (Asinara National Park): diversity and risk factors

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**Abstract.** The Sardinian coloured donkey *Equus asinus* (Perissodactyla: Equidae) and its albino colour morph represent the wildlife species most typical of the island of Asinara. This Mediterranean island represents a favourable context for ticks and tick-borne diseases; however, knowledge of the tick fauna on Asinara is scarce. A total of 106 Sardinian donkeys were inspected for tick infestation from June to November 2015. All ticks found were collected, classified by stage and sex, and identified to species level. The level of infestation of each donkey was determined; both the overall tick infestation and infestations of each detected species were classified on a scale of 1–3 to give an infestation score (IS). Overall, 256 hard ticks were collected from 60 of 106 donkeys (56.6%). *Rhipicephalus bursa*, *Haemaphysalis punctata* and *Hyalomma marginatum* (all: Ixodida: Ixodidae) infested 26.4%, 28.3% and 6.6% of donkeys, respectively. Different variables affected the IS. With reference to overall tick infestation, a higher IS was observed in donkeys grazing on grassland and Mediterranean shrubland and in albino donkeys compared with coloured donkeys. The collected ticks included species involved in the transmission of pathogens to humans, which highlights the risks for public health in a tourist destination such as Asinara National Park.

**Key words.** *Equus asinus*, *Haemaphysalis punctata*, *Hyalomma marginatum*, *Rhipicephalus bursa*, hard tick, parasites, Asinara Island.

## Introduction

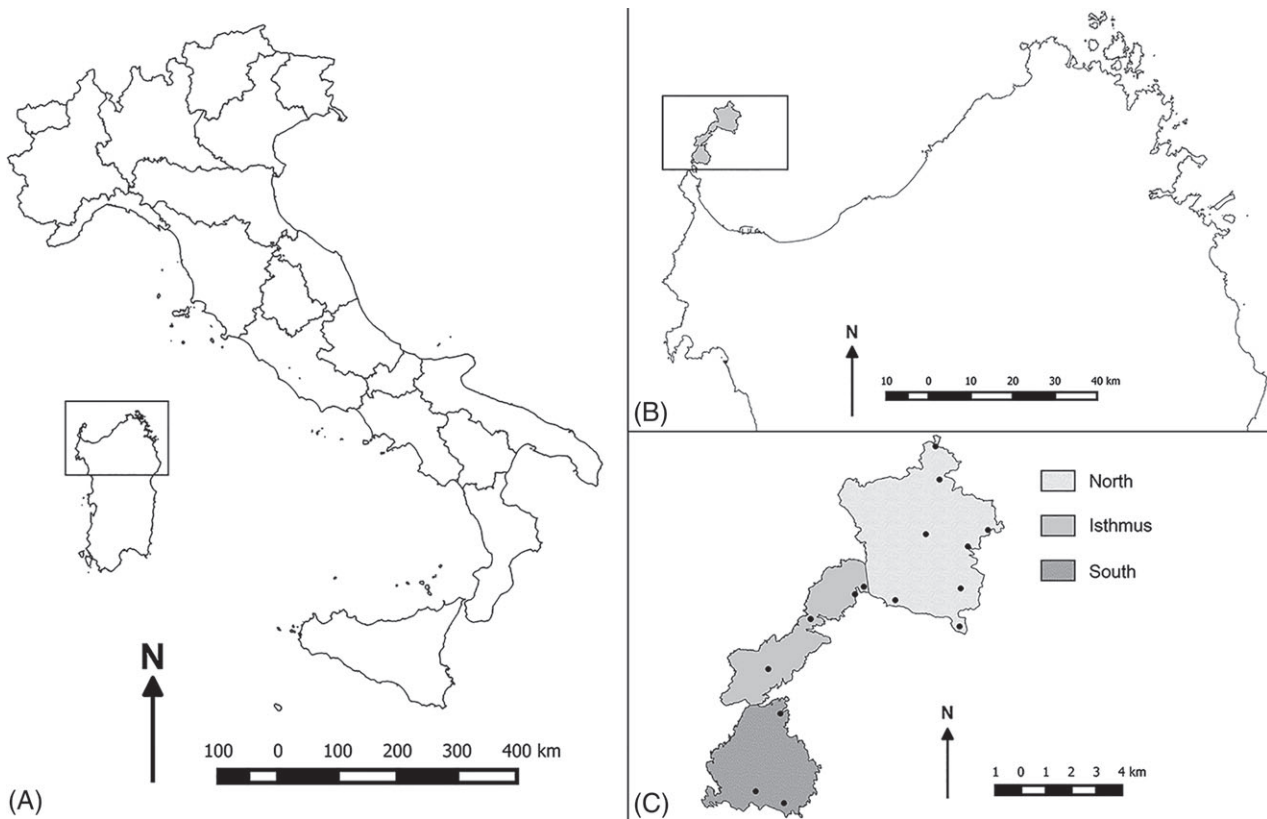
Ixodid ticks are blood-feeding arthropods with a worldwide distribution. They are the most effective vectors of a wide variety of pathogens that cause diseases in humans and animals. Ticks are also associated with irritation, painful wounds, tick bite allergies, blood loss, tick paralysis and severe toxic conditions (Sonenshine, 1993; Guglielmo *et al.*, 2014).

In nature, tick fauna and the maintenance of tick-borne pathogens depend mainly on environmental conditions, vegetation and host availability (Pfäffle *et al.*, 2013; Otranto *et al.*, 2014). Only approximately 10% of ticks feed on domesticated

animals because they are primarily parasites of wild animals, and their hosts' behaviour seems to be an essential factor in the distribution and dispersal of ticks (Estrada-Peña, 2001; McCoy *et al.*, 2001), which have only limited independent movement. Natural zones with high densities of ticks are considered to pose higher levels of risk for disease not only to animals but also to humans because ixodids are often indiscriminate in their choice of hosts and hence many may feed opportunistically on humans. In addition, ticks also reduce the recreational value of many parks, campsites and hiking paths (Olivieri *et al.*, 2017).

Asinara (41°03'28" N, 08°16'33" E) is an island in the Mediterranean Sea, located to the northwest of Sardinia, Italy.

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**Fig. 1.** Study area (A, B) showing the three regions of the island of Asinara (Asinara National Park) where ticks were collected and (C) sites of sampling of donkeys (●).

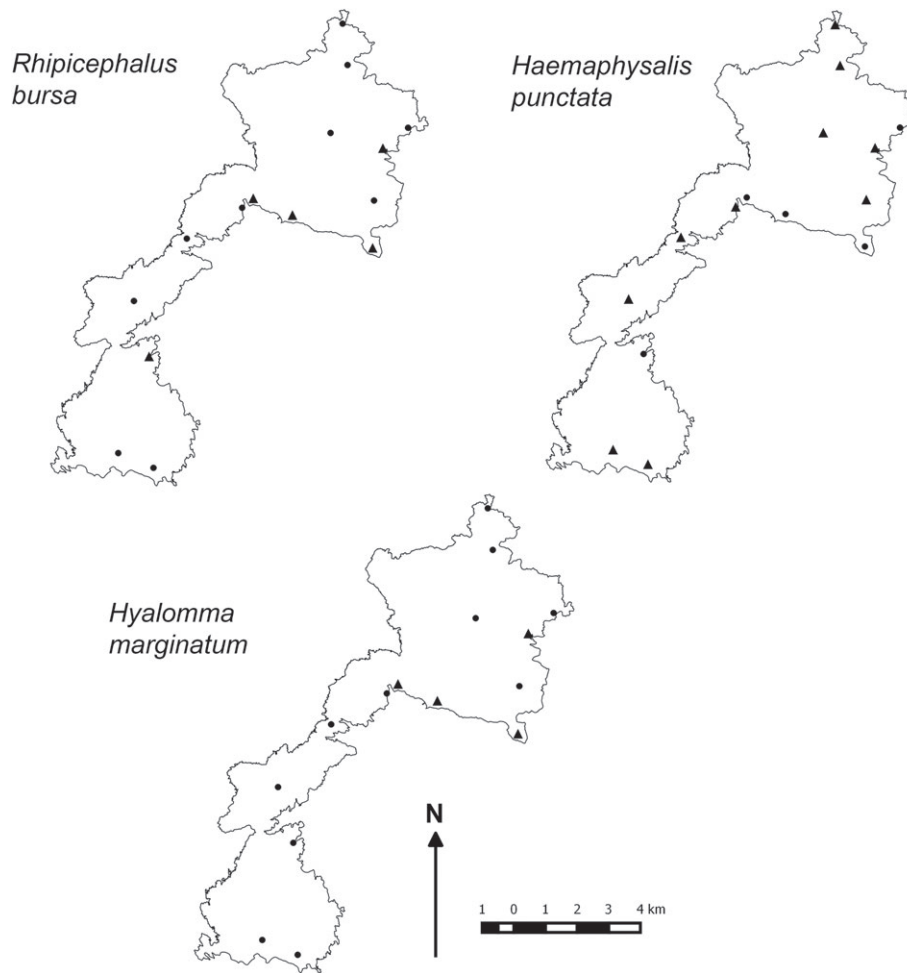
The island was inhabited until 1885, at which time it was closed and the population transferred as the island was used as a quarantine facility and subsequently a high-security prison colony. The island was opened to the public in 1999 following the formation of Asinara National Park. At present, the island is permanently inhabited by only two residents, a marshal and a sculptor. Another few dozens of workers (e.g. people employed in tourism, forest rangers, researchers) populate Asinara National Park discontinuously throughout the year. The island covers an area of 50.9 km<sup>2</sup>; its width is highly variable and ranges from 290 m at the narrowest point to 7 km at the widest. The island is mainly hilly and peaks in altitude in the northern part (408 m a.s.l.). The few flat areas stretch between the coast and the hilly areas, mainly in the southern and northern regions. The island's climate is typical of the Mediterranean region, with mild winters and hot, dry summers. The average temperature of the coldest month, February, is 10.8°C and that of the hottest month, August, is 23.3°C. Rainfall is very scarce and reaches an average of 481 mm per year.

The island is characterized by several vegetal communities and abundant wildlife. A forest grows on metamorphic rocks in the northern part of the island and micro-forests are observed in the southern part. Mediterranean shrubland is common on schist and granite rocks. Perennial and annual herbaceous species occur diffusely in grasslands. Areas covered by sparse vegetation, that are often subject to fire, are less species-rich.

The species most representative of the wildlife on Asinara island is the Sardinian coloured donkey (*Equus asinus*) and its albino colour morph. These represent some of the few wild equids still living in Europe (Kugler *et al.*, 2008). The Food and Agriculture Organization, in accordance with the register of equine and asinine Italian local breeds, classified the albino donkey as a breed of critical status with a strong risk for extinction (Scherf, 2000; Ministero delle Politiche Agricole, Alimentari e Forestali, 2013). Both donkey types live in stable groups that graze and rest together in different areas of the island. A few other species of domestic and wild ungulate are present on the island, including *Equus ferus caballus* (Perissodactyla: Equidae), *Capra hircus* (Artiodactyla: Bovidae), *Sus scrofa meridionalis* (Artiodactyla: Suidae) and *Ovis gmelini musimon* (Artiodactyla: Bovidae).

Because of its use as a high-security prison in the last century, Asinara today represents one of the largest of the Mediterranean islands that have been minimally exploited in terms of land use. As a result, the island has been little affected by human activity for many years; in this context, important factors (climatic and environmental features, wild animals) may promote both the presence of many species of hard tick and the circulation of pathogens they transmit.

Previously, knowledge about the tick fauna of Asinara island has been scarce. The last contribution of Pinna *et al.* (1996) provided information on species occurring on the island on



**Fig. 2.** Distribution of positive donkey herds in Asinara National Park by species of tick. ▲, positive sampling site; ●, negative sampling site.

different hosts. Therefore, the present data about Asinara tick fauna constitute an update. Here, the present authors report a study carried out in 2015 in Asinara donkeys with the primary aim of assessing the species and abundances of ticks infesting these donkeys. Furthermore, in order to determine the level of risk for tick bites and the transmission of tick-borne pathogens to the health of both donkeys and humans, variations in tick fauna according to space, vegetation cover and time of sampling are described and risk factors related to donkeys (donkey morph, individual features) and sampling (time, site, vegetation cover) are evaluated.

## Materials and methods

From June to November 2015, as part of a study of the genetics of the donkeys living on Asinara island, a total of 106 donkeys (38 albino and 68 coloured) belonging to 15 different stable herds sited in the isthmus and the northern and southern parts of Asinara island were inspected for tick infestation (Fig. 1). In other months of the year, the island was closed to visitors and sampling was not carried out. Donkeys were of all ages (from

4 months to 25 years) and both sexes; they grazed in different sites, characterized by three types of land cover: sparse vegetation; Mediterranean shrubland, and grassland. The donkeys were captured using tele-anaesthesia (87 donkeys) or ropes and physical handling (19 donkeys) prior to the collection of ticks. The study was approved by the appropriate committees of both Asinara National Park and the University of Sassari. The skin of each donkey was inspected for the presence of ticks. In particular, preferred feeding sites, such as the head and ears, sternum, ventral abdominal region, inguinal region, perineal region and the base of the tail, were thoroughly checked. All detected ticks were collected, preserved in 70% ethyl alcohol and classified by stage of development and sex. Then, they were identified to species level under a stereomicroscope and a light microscope according to their morphological characteristics using specific keys (Manilla, 1998). The tick infestation level of each donkey was determined for both overall tick infestation and for each detected species and classified using an infestation score (IS) on a scale of 1–3. Donkeys with no ticks, those infested with one to 10 ticks and those infested with more than 10 ticks were given scores of 1, 2 and 3, respectively. The IS was introduced as a dependent variable in generalized linear models (GLMs) with

**Table 1.** Species and prevalences of ticks collected on donkeys in Asinara National Park.

Tick species	Donkeys infested/examined, <i>n</i>	Prevalence, %
<i>Rhipicephalus bursa</i>	23/106	21.7%
<i>Haemaphysalis punctata</i>	30/106	28.3%
<i>Hyalomma marginatum</i>	2/106	1.9%
<i>R. bursa</i> and <i>H. marginatum</i>	5/106	4.7%
No ticks	46/106	43.4%

ordinal logistic regression. In each model, the following independent variables were introduced: date of sampling; location of sampling (north, south or isthmus); land cover type at the site of grazing (sparse vegetation, Mediterranean shrubland or grassland); donkey age (months); wither height (cm); body weight (kg); sex, and coat (albino or coloured). Continuous variables were tested for multicollinearity. The components of the final multivariate model (GLM) were determined by backward elimination until all remaining variables were significant ( $P < 0.05$ ). Statistical analyses were carried out using IBM SPSS Statistics for Windows Version 20.0 (IBM Corp., Armonk, NY, U.S.A.).

## Results

Overall, 256 hard ticks (111 *Rhipicephalus bursa*, 133 *Haemaphysalis punctata*, 12 *Hyalomma marginatum*) were collected from 60 of 106 donkeys (prevalence of infestation: 56.6%). *Rhipicephalus bursa*, *H. punctata* and *Hy. marginatum* infested 26.4%, 28.3% and 6.6% of donkeys, respectively; infested donkeys were found in 14 of the 15 stable groups. The spatial distribution of the positive herds is shown in Fig. 2. Monospecific infestations were observed in 55 of 60 donkeys (Table 1). The collected ticks were mainly adults (77.3%); nymphs and larvae were classified exclusively as *H. punctata* (Table 2).

In the risk factor analysis, the final models after backward elimination showed that different variables affected the IS values for overall tick infestation and *R. bursa* and *H. punctata* infestations (Table 3). With regard to overall tick infestation, a higher IS was observed in donkeys grazing on grassland [odds ratio (OR) = 5.765, 95% confidence interval (CI) 2.041–16.278;  $P < 0.01$ ] and Mediterranean brushland (OR = 2.605, 95% CI 1.088–6.241;  $P < 0.05$ ) compared with those grazing on land covered by sparse vegetation (Fig. 3). Autochthonous albino donkeys presented a higher IS (OR = 3.236, 95% CI 1.405–7.451;  $P < 0.01$ ) than coloured Sardinian donkeys (Fig. 4). All of the other individual variables (age, wither height, body weight, sex) introduced in the GLM produced non-significant results; the same was observed for location (north, isthmus, south) and date of sampling. Infested donkeys were found throughout the territory of Asinara island and throughout the study period (Fig. 5A). *Rhipicephalus bursa* IS was related to the date of sampling: both the prevalence and level of infestation in donkeys were higher in June and July than in October and November (Fig. 5B). With regard to individual features, the risk for a higher *R. bursa* IS increased in older animals (OR = 1.009, 95% CI 1.002–1.016;  $P < 0.05$ ).

**Table 2.** Tick species collected on donkeys in Asinara National Park stratified by tick stage.

Tick species	Adults		Nymphs	Larvae	Total	
	Males	Females			<i>n</i>	%
<i>Rhipicephalus bursa</i>	57	54	0	0	111	43.4%
<i>Haemaphysalis punctata</i>	19	56	31	27	133	52.0%
<i>Hyalomma marginatum</i>	7	5	0	0	12	4.7%
Total	83	115	31	27	256	

Date of sampling was a predictor of *H. punctata* IS (Fig. 5C); the only individual feature positively related to *H. punctata* IS in donkeys was the albino coat (OR = 5.863, 95% CI 1.822–18.862;  $P < 0.01$ ). Despite the small number of infested donkeys, date of sampling and land cover type were significant predictors of *Hy. marginatum* IS in the final model after backward elimination.

## Discussion

This study presents data on the species of adult ticks collected on donkeys living on Asinara island and their occurrence according to time, vegetation cover and site of sampling. Previous studies on the ixodofauna of the island showed that albino donkeys were infested by *Hyalomma excavatum*, *R. bursa*, *Dermacentor marginatus* (Ixodida: Ixodidae), *H. punctata*, *Haemaphysalis otophyla* and *Rhipicephalus sanguineus* (Garippa & Sanna, 1990; Pinna *et al.*, 1996). The current survey confirms the presence of *H. punctata* and *R. bursa*, but not the presence of the other ticks previously found. Furthermore, the donkeys sampled in 2015 were infested by *Hy. marginatum*, which has not been recorded previously in donkeys living on Asinara island.

*Haemaphysalis punctata* was the predominant species in Asinara's donkeys and was especially abundant in albino animals. Torina *et al.* (2006) recorded this tick in donkeys from Sicily, but at a lower frequency (4.3%) than that found in donkeys on Asinara island (52.0%). Records of this species on donkeys are scarce, but its presence on horses in central and southern Italy has been reported (Khouri *et al.*, 1994).

*Rhipicephalus bursa* was the second most frequently found species on donkeys; it was also found in donkeys from Sicily (21.4%) (Torina *et al.*, 2006). Furthermore, in horses from Corsica, *R. bursa* had a moderate prevalence (21.1%) (Grech-Angelini *et al.*, 2016). *Rhipicephalus bursa* appeared to be a predominant species on domestic ruminants in other surveys carried out on the islands of Sardinia, Sicily and Corsica (Garippa *et al.*, 1998; Torina *et al.*, 2006; Grech-Angelini *et al.*, 2016).

*Hyalomma marginatum* accounted for very few of the total collection of specimens; however, the study confirms the presence of feeding adults of *Hy. marginatum* on Asinara and demonstrates that the tick is able to complete its lifecycle on the

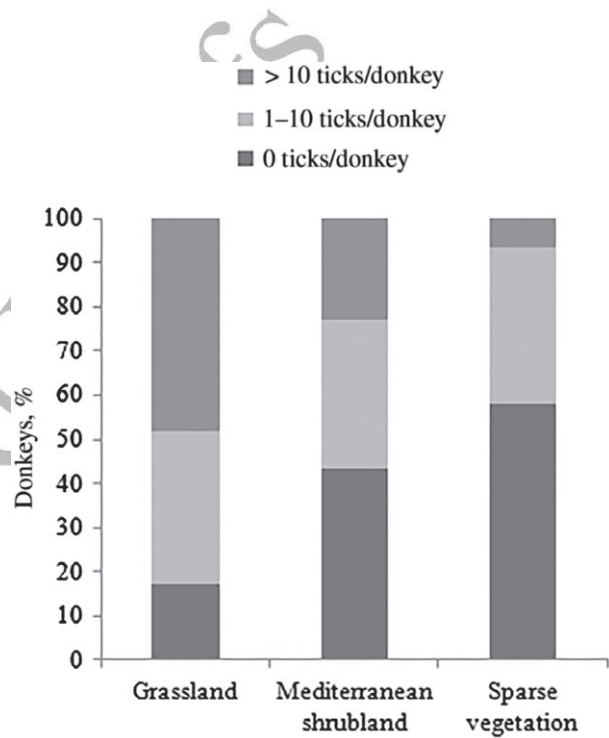
**Table 3.** Risk factors associated with tick infestation levels in donkeys on Asinara island. Final models with ordinal logistic regression after backward elimination.

Dependent variable	Independent variable	Factor	Odds ratio	95% CI	P-value	
Overall tick infestation	Land cover type	Grassland	5.765	2.041–16.278	< 0.01†	
		Mediterranean brushland	2.605	1.088–6.241	< 0.05*	
		Sparse vegetation (reference)	1			
<i>Rhipicephalus bursa</i> infestation	Donkey coat	Albino	3.236	1.405–7.451	< 0.01†	
		Coloured (reference)	1			
<i>Rhipicephalus bursa</i> infestation	Date of sampling	(continuous variable)	0.999	0.999–0.999	< 0.001‡	
	Donkey age	(continuous variable)	1.009	1.002–1.016	< 0.05*	
<i>Haemaphysalis punctata</i> infestation	Date of sampling	(continuous variable)	1.000	1.000–1.000	< 0.05*	
	Donkey coat	Albino	5.863	1.822–18.862	< 0.01†	
<i>Hyalomma marginatum</i> infestation	Date of sampling	(continuous variable)	0.999	0.999–0.999	< 0.05*	
		Land cover type	Grassland	15.459	2.150–111.132	< 0.01†
			Mediterranean shrubland	26.281	0.905–763.522	0.057
		Sparse vegetation (reference)	1			

Significant at \* $P < 0.05$ ; † $P < 0.01$ ; ‡ $P < 0.001$ .  
CI, confidence interval.

island. The immature stages of *Hy. marginatum* are parasites of small and medium-sized mammals and birds, whereas the adult stages primarily feed on ungulates. Findings of ticks on migratory birds suggest that *Hy. marginatum* could be introduced into new areas by avian hosts and that its survival may be influenced by climatic conditions. According to Estrada-Peña *et al.* (2015), the study area is included in the Mediterranean south climate domain, which represents suitable conditions for this tick. *Hyalomma marginatum* appears to be very common in Corsica (Grech-Angelini *et al.*, 2016). It was also recovered in other Mediterranean islands with similar climatic conditions, such as Menorca, Sicily and Sardinia (Garippa *et al.*, 1998; Castellà *et al.*, 2001; Torina *et al.*, 2006). On the island of Sardinia, the tick was previously observed at a higher prevalence on cattle (40%) than on horses (16%) (Deiana & Arru, 1960). By contrast, other studies have confirmed that equids are suitable hosts for the tick; in Corsica, the proportion of *Hy. marginatum* observed on horses (78%) was much higher than that found on cattle (26%) (Grech-Angelini *et al.*, 2016). *Hyalomma marginatum* was also reported as the most common tick species among Italian horses, although the overall prevalence of tick infestation was low (Lori *et al.*, 2010).

The diversity of the tick species found on donkeys from Asinara also appears to be low in comparison with the findings of studies carried out in other areas; in Sicily, Torina *et al.* (2006) recorded six tick species and, more recently, Horak *et al.* (2017) found donkeys infested with eight tick species. Adults of the three species of tick identified on Asinara and on coloured donkeys in the current study usually feed on larger mammals, such as livestock and wild ungulates, and, according to Estrada-Peña *et al.* (2013), ruminants are suitable hosts for adult ticks of *H. punctata*, *R. bursa* and *Hy. marginatum*. A possible hypothesis that may explain the predominance of these ticks and the low species richness of ticks on donkeys in the present study concerns the dramatic reduction in the number of domestic ruminants on Asinara from 1998 onward, when the jail went out of use. At the end of 1987, when Asinara island



**Fig. 3.** Tick infestations in donkeys in Asinara National Park by vegetation coverage type.

was used as a prison, the numbers of ruminants amounted to 411 cattle, 1760 sheep and 1128 goats (G. Garippa, unpublished data, YEAR). At the time, horses ( $n = 130$ ), pigs ( $n = 278$ ) and unknown numbers of wild goats, mouflons and wild boars were also present. Currently, only a flock of almost 100 domestic goats grazes in a fenced pasture. However, wild goats, horses, mouflons and wild boars continue to roam freely on the island as in the past. In addition, as demonstrated by previous studies, two of the three species of tick (*H. punctata* and *R. bursa*) identified on Asinara donkeys usually prefer to feed on domestic ruminants

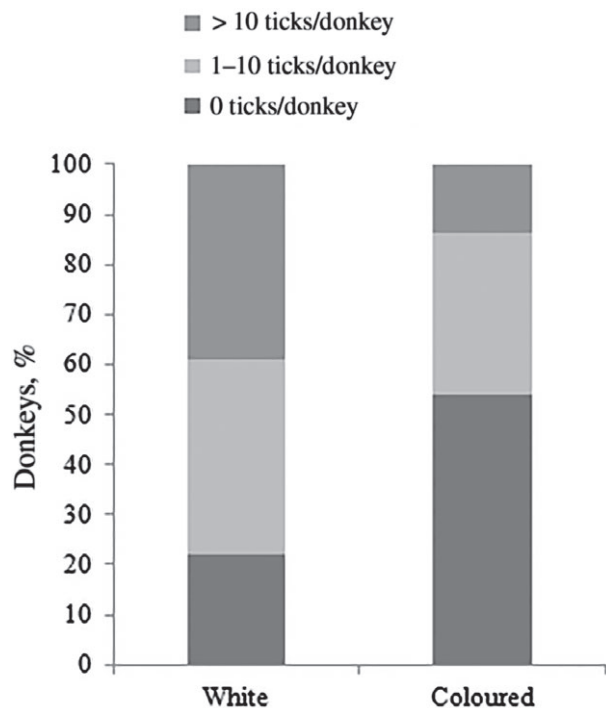


Fig. 4. Tick infestations in donkeys in Asinara National Park by colour of donkey.

rather than on equids (Psaroulaki *et al.*, 2006; Torina *et al.*, 2006; Sofizadeh *et al.*, 2014). When the jail was in use, dogs and cats also lived on the island; at present, there are few domestic and sylvatic carnivores in Asinara National Park, which possibly contributes to the low acarological diversity in Asinara donkeys.

All of the ticks identified were restricted in range to the Mediterranean area, particularly to the biome 'Mediterranean forests, woodlands and scrub', and occurrences vary according to ecological region (Estrada-Peña *et al.*, 2013). Given that in the study area, there were no donkeys grazing in forest or woodland, the highest occurrence of tick infestation in animals grazing in Mediterranean shrubland and grassland appears to be grossly in accordance with the findings of Estrada-Peña *et al.* (2013). The sparse vegetation is probably not suitable for free-living stages of ticks. Differences in tick occurrence may also be linked to life stage and host. In the present study, mainly adult ticks were recovered (77.3%); larval and nymphal stages may be more strictly located in forests and the forest-scrub interface, which favours the presence of small mammals and birds. Moreover, decaying vegetation in the forest may provide higher levels of humidity which support the survival and moulting of immature stages, whereas more resistant adult questing ticks may reach more exposed areas in their efforts to find ungulate hosts.

Within the study period, date of sampling was not a significant predictor of overall tick infestation in Asinara donkeys. Thus, in this timeframe, the risk for tick bites seemed to be uniform in late spring/early summer and autumn; by contrast, in the final models for the three species of tick considered separately, date of sampling was always a significant predictor of IS. All *H. punctata* individuals of all stages were detected on

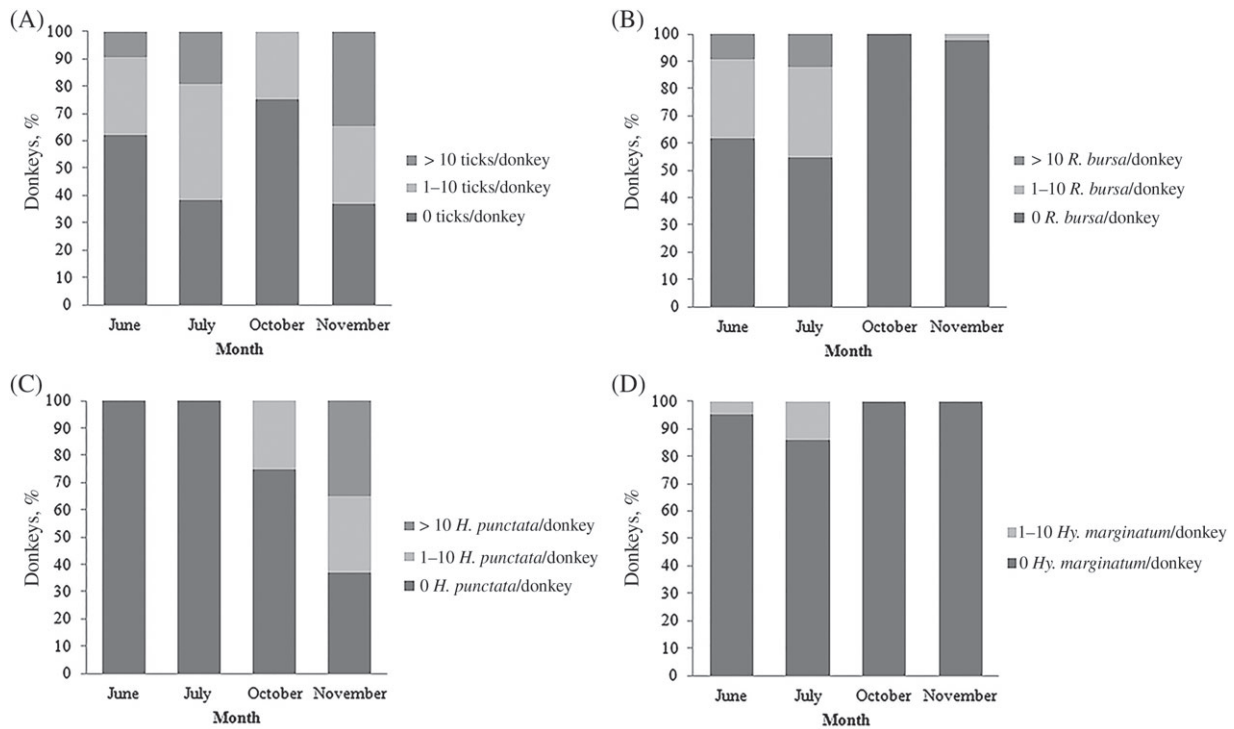
donkeys in autumn; this is in accordance with other surveys on Mediterranean islands (Torina *et al.*, 2006; Grech-Angelini *et al.*, 2016). Hence, taking into account the seasonal dynamics of the various ticks, the lack of recording on equids in previous surveys may have depended on sampling carried out at times when the tick was not active (Psaroulaki *et al.*, 2006; Sofizadeh *et al.*, 2014). The opposite trend was observed for *R. bursa* and *Hy. marginatum*; both species were collected from donkeys mainly (*R. bursa*) or exclusively (*Hy. marginatum*) in late spring/early summer, similarly to collections reported by Torina *et al.* (2006), Sofizadeh *et al.* (2014) and Grech-Angelini *et al.* (2016).

With respect to risk factors for donkeys, albino donkeys were more highly infested than coloured donkeys. Albinism predisposes to skin diseases and ophthalmological problems: dermatitis and keratoconjunctivitis linked to photosensitivity have been described in Asinara albino donkeys. This may also result in a lower resistance to ectoparasitic infestation, as observed for myiasis cutanea (Pinna *et al.*, 1996); moreover, low visual capacity causes albino donkeys to shelter from excessive light in the sunny hours and their prolonged presence near trees and shrubs may result in greater exposure to ticks in comparison with coloured animals.

With respect to the vectorial roles of ticks collected from donkeys on Asinara island, it should be noted that *H. punctata* is one of the most commonly reported species of this genus found on humans; it is confined to forest habitats in Europe and is mainly involved in the transmission of tick-borne encephalitis (TBE) virus, for which it is a competent vector. *Apodemus flavicollis* (Rodentia: Muridae) and *Myodes glareolus* (Rodentia: Cricetidae), the two species of rodent considered to represent the major reservoirs for the circulation of TBE virus in Central Europe, are not reported on the island and hence there should be no risk for TBE infection of humans within Asinara National Park. However, *H. punctata* is also a vector of *Coxiella burnetii* (Legionellales: Coxiellaceae) and *Rickettsia slovaca* (Rickettsiales: Rickettsiaceae) (Estrada-Peña & Jongejan, 1999).

Evidence of the presence of *Hy. marginatum* and *R. bursa* in the study area is of particular interest in view of the roles of these tick species in the transmission of, respectively, *Theileria equi* (Piroplasmida: Theileriidae) and *Babesia caballi* (Piroplasmida: Babesiidae), the agents of equine piroplasmosis (Rothschild, 2013). Consequently, the exposure of Asinara donkeys to these vectors poses a risk for infection with tick-borne disease (babesiosis, theileriosis) that may worsen their status as an endangered species. Although babesiosis and theileriosis in equids can be acute, subacute or chronic, piroplasmosis in endemic areas is frequently subclinical. Infected animals may remain carriers for long periods and act as sources of infection for other ticks. The poor management, inadequate veterinary care and poor nutrition common to equine populations living in natural and protected reserve areas may exacerbate the impacts of infection (Scoles & Ueti, 2015).

Furthermore, the finding of *Hy. marginatum* is noteworthy as it is the competent vector and reservoir of *Rickettsia aeschlimannii*, a spotted fever rickettsia recently associated with human cases of Mediterranean spotted fever-like disease (Germanakis *et al.*, 2013). High rates of infection by *R. aeschlimannii* have been found previously in *Hyalomma* ticks



**Fig. 5.** Tick infestations in donkeys in Asinara National Park by month of sampling: (A) all ticks; (B) *Rhipicephalus bursa*; (C) *Haemaphysalis punctata*; (D) *Hyalomma marginatum*.

in Sardinia (Mura *et al.*, 2008), Corsica (Matsumoto *et al.*, 2004) and other Mediterranean regions (Beninati *et al.*, 2005; Tomasone *et al.*, 2013; Scarpulla *et al.*, 2016). *Hyalomma marginatum* frequently bites humans, as described by Matsumoto *et al.* (2004). This suggests a potentially high risk for human exposure to rickettsia infection in Asinara National Park because the island is frequented by many visitors; moreover, the vector may also harbour several other pathogens (e.g. Crimean–Congo haemorrhagic fever virus, West Nile virus and Thogoto virus).

## Conclusions

This study carried out in Asinara National Park showed that albino donkeys are more exposed to tick infestations than Sardinian coloured donkeys. The collected ticks included species involved in the transmission of pathogens to humans and, although further studies are required to exhaustively characterize the ixodofauna of the island, the data obtained in the present work show the risks to public health in the context of Asinara as an important tourist destination. Visitors engaged in tourism and outdoor recreational activities (trekking, bicycling and hiking) are at risk for exposure to tick bites and the transmission of pathogens (Colwell *et al.*, 2011).

In summary, the data collected in the present study further emphasize that the preservation of natural reserves may create conditions favourable for tick survival; indeed, measures to protect wild fauna, combined with management practices designed to preserve the natural environmental, increase the ecological

niches and proliferation of hosts crucial to the maintenance of both ticks and zoonotic pathogens (Estrada-Peña, 2009; Randolph, 2009). Thus, it is necessary to provide information on these issues to people visiting protected areas in order to prevent tick bites and related sanitary risks.

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The authors declare no conflicts of interest.

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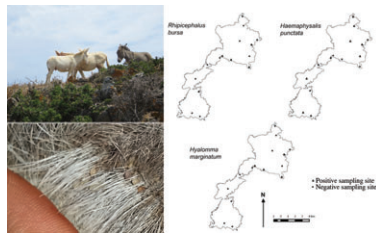
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## Graphical Abstract

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Sardinian coloured and albino donkeys are the most representative animals of Asinara National Park, which is visited yearly by thousands of tourists. *Rhipicephalus bursa*, *Hyalomma marginatum* and *Haemaphysalis punctata* were collected from donkeys on Asinara. The tick species detected are competent vectors of several pathogens and thus pose a health risk to humans and animals.