Veterinary Microbiology 251 (2020) 108910

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Distribution and risk factors of canine haemotropic mycoplasmas in hunting dogs from southern Italy

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ARTICLE INFO

Keywords:
Mycoplasma haemocanis
Candidatus mycoplasma haematoparvum
Hunting dogs
Italy

ABSTRACT

Mycoplasma haemocanis (Mhc) and "*Candidatus* Mycoplasma haematoparvum" *(CMhp)* are the main haemoplasma species known to infect dogs. The aim of this study was to determine the prevalence of haemoplasma species infections in hunting dogs from southern Italy and assess related risk factors. 1,433 hunting dogs living in Campania region were tested by qPCR assay. The prevalence was 19.9 %; 13.1 % for *Mhc* and 11.4 % for *CMhp*; 4.6 % showed a coinfection with both haemoplasma species. Statistical analysis revealed living in Salerno province (*Mhc*: OR 3.72; *CMhp*: OR 2.74), hound (*Mhc*: OR 5.26; *CMhp*: OR 8.46) and mixed breed (*Mhc*: OR 3.38; *CMhp*: OR 2.80), rural environment (*Mhc*: OR 12.58; *CMhp*: OR 10.38), wild mammal hunting (*Mhc*: OR 8.73; *CMhp*: OR 8.32), cohabitation with other animals (*Mhc*: OR 2.82; *CMhp*: OR 2.78) and large pack size (*Mhc*: OR 2.96; *CMhp*: OR 1.61) as risk factors for haemoplasmas. Male gender (OR 1.44) and tick infestation history (OR 1.40) represented risk factors only for *Mhc*, while adult age (2–7 years - OR 2.01; > 7 years - OR 1.84) and large body size (OR 1.48) were associated only to *CMhp*. *Mhc* infection was significantly associated to *Babesia vogeli* ($p < 0.05$) and *Hepatozoon canis* ($p < 0.001$), while *CMhp* with *H. canis* ($p < 0.001$). This study adds information on haemoplasma species distribution in hunting dogs in southern Italy. Outdoor lifestyle and contact with wild fauna, through greater exposure to tick infestation, or possibly wounds acquired during hunting or fighting, could be factors contributing to haemoplasma infections.

1. Introduction

Haemotropic mycoplasmas, also known as haemoplasmas, are unculturable small, cell wall-deficient bacteria that reside on the surface of erythrocytes (Sykes and Tasker, 2014). Two main species of haemoplasmas infect dogs worldwide: *Mycoplasma haemocanis* (*Mhc*) (Messick, 2004) and "*Candidatus* Mycoplasma haematoparvum" (*CMhp*) (Sykes et al., 2004). Other haemoplasma species have been found occasionally in dogs. "*Candidatus* Mycoplasma haemominutum", a feline haemoplasma species, was reported in dogs from China and Japan (Zhuang et al., 2009; Obara et al., 2011). In Australia "*Candidatus* Mycoplasma haemobos" and a novel haemoplasma species, that showed a high similarity with the haemofelis group of haemoplasmas, were detected in dogs from Aboriginal communities (Barker et al., 2012; Hii et al., 2012). Furthermore, Varanat et al. (2011) reported the presence of *Mycoplasma ovis* DNA in splenic hemangiosarcoma specimens surgically obtained from dogs.

Although incompletely studied, both haemoplasmas may cause acute haemolytic anaemia and potentially contribute to chronic diseases in dogs (Messick, 2004). In addition, both species have been reported to be potential zoonotic agents (Maggi et al., 2013a,b). The natural route of transmission to the dog remains unknown, although some modalities have been hypothesized. Bloodsucking arthropods may be involved in the transmission of canine haemoplasmas. In particular, the brown dog tick, *Rhipicephalus sanguineus* sensu lato, is likely to play a role as a vector and reservoir for *Mhc* (Seneviratna et al., 1973). In Europe, the brown dog tick is commonly encountered in areas with Mediterranean climate and the high prevalence of canine haemoplasma infections

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https://doi.org/10.1016/j.vetmic.2020.108910
Received 26 July 2020; Accepted 25 October 2020
Available online 1 November 2020

found in these countries may support the hypothesis of its vector role (Novacco et al., 2010). Transplacental transmission, is another proposed modality (Lashnits et al., 2019). Finally, biting and fighting are considered a possible route of haemoplasma transmission. In particular, the prevalence of *Mhc* was higher in Japanese fighting dogs compared to other breeds (Sasaki et al., 2008) and, this finding was attributed to direct transmission of *Mhc* via infected blood during aggressive contact (Willi et al., 2010). In cats, blood transfusions have been reported as a source of "*Candidatus* Mycoplasma haemominutum" infections (Gary et al., 2006), but currently we are not aware of published reports of blood transfusion transmission among dogs.

Canine haemoplasma infections are most often asymptomatic; however, serious or fatal disease can occur in association with immunosuppression or co-infection with other pathogens. In some populations young animals and male dogs were found to be more susceptible to infection than adult and female dogs (Novacco et al., 2010). Haemoplasma infections are usually chronic and subclinical in immunocompetent dogs, but immunosuppression may lead to haemolytic anaemia in association with splenectomy, concurrent infectious diseases, immunosuppressive drug administration, or neoplasia (Kemming et al., 2004; Sykes et al., 2004; Willi et al., 2010). Moreover, the presence of demodectic mange was associated with canine haemoplasmosis (Novacco et al., 2010). These mites may play a role in the mechanical transmission of haemoplasmas, or this parasitic skin disease may signal a compromised immune system in the affected animals.

Due to the specific culture requirements, which have not yet been fully established, the identification of haemoplasmas in routine practice has traditionally been based on their visualization on Giemsa-stained blood smears in association with typical clinical symptoms of haemolytic anaemia. However, cytological methods have low sensitivity and specificity and so now the polymerase chain reaction (PCR), both conventional and quantitative (qPCR), is considered the gold standard method for haemoplasma detection and species differentiation (Wengi et al., 2008; Gentilini et al., 2009). Serological assays are not yet routinely available.

Mhc and *CMhp* seem to exhibit worldwide distribution, but only limited molecular prevalence data are available. In Europe, a higher prevalence was reported in countries with Mediterranean climate when compared to Switzerland (Wengi et al., 2008; Novacco et al., 2010). In Italy, data on haemoplasma infections in dogs are limited, especially in the south of the country. The infection rates in dogs with different lifestyles, sampled in three urban areas throughout the peninsula, was 7.5 % in northern Italy, 9.5 % in central Italy and 11.5 % in Sicily (Novacco et al., 2010). Ravagnan et al. (2017) reported the prevalence of haemoplasmas in 395 dogs, including 117 blood donor candidate owned dogs and 278 free-roaming dogs from northern and north-eastern Italian provinces. The overall prevalence in this latter survey was 4.5 % (18/395) with a 6.1 % (17/278) prevalence in free-roaming dogs compared to 0.8 % (1/117) among blood donor candidates. Both *Mhc* and *CMhp* were identified in the free-roaming dogs (*Mhc* in the blood donor) and no significant association was found between haemoplasma infection and gender, age or living area.

In addition, no published data are available as to whether hunting dogs may be at greater risk for haemoplasma infections, compared to other dogs (e.g. household dogs). Hunting dogs are at increased risk of tick infestations due to closer contact among pack dogs and more frequent exposure to wooded and rural areas, as previously reported for other known vector-borne pathogens (*Babesia*, *Ehrlichia*, *Anaplasma*) (Piantedosi et al., 2017; Veneziano et al., 2018).

The purpose of this study was to determine the molecular prevalence of haemoplasmas in hunting dogs from Southern Italy, where *R. sanguineus* s.l. is a common vector for other major haemoparasites, such as *Ehrlichia canis* and *Babesia vogeli*. We also investigated potential risk factors associated with haemoplasma PCR positive status.

2. Materials and methods

2.1. Dogs and sampling

The study included 1,433 healthy hunting dogs from 153 municipalities, representative of three study provinces in the Campania region of southern Italy. The study was conducted as a component of the hunting dog's health assistance program of University of Naples, which was supported by the Italian management committees of the respective hunting districts (ATC). The study was approved by the Ethical Animal Care and Use Committee of the University of Naples "Federico II" (Approval number: 0039904; date of approval 20 October 2014) and, written consent was obtained from the owners of the hunting dogs.

The study area had surface of 5,698.81 square km, including the hunting district of Naples (ATC NA), Avellino (ATC AV) and one of the two hunting districts of Salerno (ATC SA 1). These are located in southern Italy in the provinces of Naples (40° 50′ N - 14° 15′ E), Avellino (40° 54′ 55″ N - 14° 47′ 22″ E) and Salerno (40° 41′ 00″ N - 14° 47′ 00″ E).

The necessary sample size to estimate prevalence was calculated using the formula proposed by Thrusfield (1995) considering the following epidemiological data: expected molecular prevalence of 4.5 % for haemotropic mycoplasmas based on the results of a recent similar study in canine populations from Northern Italy (Ravagnan et al., 2017); confidence interval (99 %) and desired absolute precision (2%), based on the number of hunters in Campania region (n° 38,611 hunters in the season 2014–2015 and assuming a dog for each hunter) (BURC, 2019).

Blood samples were collected in 44 private veterinary hospitals located in the study area between March and October 2015. Sampling was performed by different veterinary operators during a routine health check.

Ten milliliters of blood collected by jugular venipuncture after 12 h of fasting were placed into tubes containing potassium ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. The samples were stored at −80 °C and defrosted immediately before batch analysis.

A questionnaire was submitted to each owner to obtain information about the dog's province residence, breed category, body size (small, medium, large) as indicated by the Italian Kennel Club (ENCI, 2020), age (< 2 years, 2–7, > 7 years), gender, pack size when cohabiting with other dogs, contact with other pet or farm animals (dogs, cats, horses and ruminants), type of hunting (birds or wild mammals), living environment (rural or urban area), history of tick infestation and ectoparasite control practices (frequency of ectoparasiticide treatments).

2.2. Molecular assay

Mhc and *CMhp* quantitative PCR (qPCR) was performed after nucleic acid extraction from EDTA-anti-coagulated blood samples at a commercial laboratory as part of a broad screening panel for vector-borne pathogens (Tick/Vector Comprehensive RealPCR™ Panel Canine, IDEXX Laboratories, Inc.). Concurrently with the screening panel, quality control assays including a quantitative PCR-positive control, PCR-negative control, internal positive control spiked into lysis solution, quantitative DNA internal sample quality control targeting the host 18S rRNA, negative extraction control and environmental contamination monitoring control were performed. The target sequence for *Mhc* and *CMhp* species-specific qPCR was the 16S rRNA gene (GenBank Accessions AF197337 and AY383241, respectively) similar to previously published assays for related pathogens in cats (Sykes et al., 2008).

Babesia vogeli, Babesia canis, Hepatozoon canis and *Ehrlichia canis* qPCR were performed as previously described (Veneziano et al., 2018; Pacifico et al., 2020).

2.3. Statistical analysis

Statistical analysis for *Mhc* and *CMhp* PCR-positive was performed by

using a contingency table on Fisher's exact test. Odds ratios (OR) and confidence interval (CI) were estimated by the coefficient of the logistic regression. P-value < 0.05 was considered as the threshold for statistical significance.

3. Results

The overall PCR-positive rates were 13.1 % (188/1,433; 95 % CI 6.85–12.06; OR 9.09) for *Mhc* and 11.4 % (164/1,433; 95 % CI 6.58–11.89; OR 8.84) for *CMhp*. Coinfection with both haemoplasma species was found in 4.6 % (67/1433) of dogs. The distribution of *Mhc* and *CMhp* PCR-positive dogs in the study area is shown in Fig. 1. The proportion of *Mhc* and *CMhp* PCR-positive hunting dogs in relation with different variable are summarized in Table 1, while the potential risk factors associated with haemoplasma infection are showed in Table 2.

Statistical analysis revealed living in Salerno province (*Mhc*: OR 3.72, 95 % CI 2.65–5.21; *CMhp*: OR 2.74, 95 % CI 1.94–3.86), hound (*Mhc*: OR 5.26, 95 % CI 3.76–7.36; *CMhp*: OR 8.46, 95 % CI 5.69–12.56), mixed breed (*Mhc*: OR 3.38, 95 % CI 1.91–5.98; *CMhp*: OR 2.80, 95 % CI 1.52–5.16), rural environment (*Mhc*: OR 12.58, 95 % CI 1.68–87.99; *CMhp*: OR 10.38, 95 % CI 1.43–75.21), wild mammal hunting (*Mhc*: OR 8.73, 95 % CI 5.84–13.05; *CMhp*: OR 8.32, 95 % CI 4.91–14.10) cohabitation with other animals (*Mhc*: OR 2.82, 95 % CI 1.36–5.86; *CMhp*: OR 2.78, 95 % CI 1.27–6.05) and large pack size (> 10 dogs) (*Mhc*: OR 2.96, 95 % CI 1.99–4.40; *CMhp*: OR 1.61, 95 % CI 1.06–2.44) as associated risk factors for the haemoplasma species, regardless of dual infection. Male gender (OR 1.44, 95 % CI 1.05–1.97) and tick infestation history (OR 1.40, 95 % CI 1.02–1.91) represented risk factors for all dogs infected by *Mhc*, while adult age (2–7 years - OR 2.01, 95 % CI 1.33–3.05; > 7 years - OR 1.84, 95 % CI 1.10–3.08) and large body size (OR 1.48, 95 % CI 1.02–2.14) were variables significantly associated with all dogs infected by *CMhp*.

Concurrent detection of *B. vogeli* (8/188; 4.3 %; 95 % CI 1.40–7.29; p < 0.05) and *H. canis* (49/188; 26.1 %; 95 % CI 19.8–32.4; p < 0.001) DNA was significantly associated with *Mhc* positive animals, regardless of dual infections. In contrast, *H. canis* presence was significantly associated with *CMhp* PCR positive status (45/164; 27.4 %; 95 % CI 20.6–34.2; p < 0.001).

4. Discussion

Mhc and *CMhp* have been described in dogs throughout the world (Roura et al., 2010; Rani et al., 2011; Compton et al., 2012; Hii et al., 2015), but few data are available on haemoplasma infections in dogs in Italy. To the authors' knowledge, this is the first large-scale molecular survey on *Mhc* and *CMhp* infections in dogs in southern Italy. In our study the PCR-positivity rates (13.1 % and 11.4 % for *Mhc* and *CMhp*, respectively) were higher than those found by Novacco et al. (2010) in dogs from urban areas sampled in northern (7.5 %) and central Italy (9.5 %), but similar to those reported by the same authors in Sicily, southern Italy (11.5 %). Ravagnan et al. (2017) showed in northern Italy a very low prevalence in privately owned candidate blood donors (0.8 %) as compared to free-roaming dogs (6.1 %). Other studies performed in Europe revealed a marked difference in prevalence in dogs among countries, ranging from 1.2%–40% (Wengi et al., 2008; Novacco et al., 2010; Roura et al., 2010; Ravagnan et al., 2017; Aktas and Ozubek, 2018; Hofmann et al., 2019). These discrepancies can be explained by differences in geographical areas, the characteristics of dog populations tested, and variability of the molecular methods used for haemoplasma detection.

It has been proposed that climate could play an important part in the canine haemoplasma epidemiology, with an increased risk in warmer countries, such as those in Mediterranean areas, potentially in relation to the greater abundance of the potential arthropod vector, *R. sanguineus* s.l., in these areas (Willi et al., 2010). Our results showed a significant association between history of tick infestation and *Mhc* infection in the

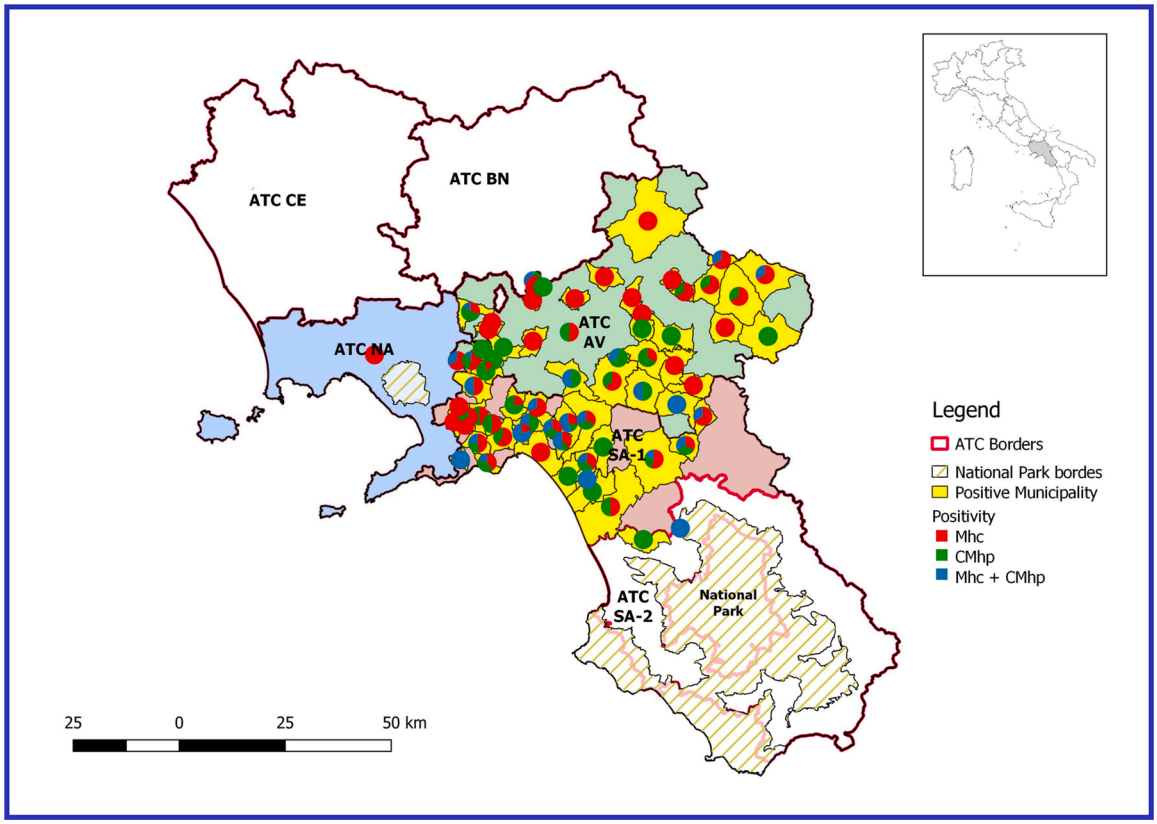

Fig. 1. Distribution map of canine haemotropic mycoplasmas PCR-positive hunting dogs in the study area.

Table 1
PCR prevalence (%) and confidence interval (95 %) of *Mhc and CMhp* in hunting dogs in southern Italy.

Variable	N° of dogs tested	*Mhc*		*CMhp*	
		N° positive (%)	95 %CI	N° positive (%)	95 %CI
Province					
Salerno	641	135 (21.1)	17.9 – 24.3	109 (17.0)	14.1–19.9
Avellino	552	52 (9.4)	7.0 – 11.8	55 (10)	7.5–12.5
Napoli	240	1 (0.4)	0 – 1.2	0 (0)	–
Breed category					
Shepherd	1	1 (100)	–	0 (0)	–
Terrier	11	2 (18.2)	0 – 41.0	1 (9.1)	0 – 26.1
Hound	525	133 (25.3)	21.6 – 29.0	130 (24.8)	21.1–28.5
Pointing	821	32 (3.9)	2.6 – 5.2	16 (1.9)	1.0–2.8
Mixed-breed	59	19 (32.2)	20.03 – 44.1	15 (25.4)	14.3–36.5
Retrievier	10	0 (0)	–	0 (0)	–
NA	6	1 (16.7)	0 – 46.5	2 (33.3)	0 – 71.0
Body size					
Small	24	4 (16.7)	1.8 – 318.6	2 (8.3)	0 – 19.3
Medium	1095	146 (13.3)	11.3 – 15.3	115 (10.5)	8.7–12.3
Large	310	37 (11.9)	8.3 – 15.5	46 (14.8)	10.8–18.8
NA	4	1 (25.0)	0 – 67.4	1 (25.0)	0 – 67.4
Age Years					
<2	311	40 (12.9)	9.2 – 16.6	9 (2.9)	1.0–4.8
2–7	1008	132 (13.1)	11.0 – 15.2	134 (13.3)	11.2–15.4
>7	109	15 (13.8)	7.3 – 20.3	20 (18.3)	11.0–25.6
NA	5	1 (20)	0 – 55.1	1 (20)	0– 55.1
Gender					
Female	642	70 (10.9)	8.5 – 13.3	66 (10.3)	7.9–12.7
Male	789	118 (15.0)	13.3 – 16.7	97 (12.3)	10.8–13.8
NA	2	0 (0)	–	1 (50.0)	47.6–52.3
Pack size					
1	122	7 (5.7)	4.6 – 6.8	6 (4.9)	3.9 – 5.9
<10	1148	139 (12.1)	10.2 – 14.0	123 (10.7)	8.9–12.5
>10	152	42 (27.6)	20.5 – 34.7	33 (21.7)	15.1–28.3
NA	11	0 (0)	–	2 (18.2)	0 – 41.0
Cohabitation with other animals					
No	143	8 (5.6)	1.8 – 9.4	6 (4.2)	0.9 – 7.5
Yes	1284	180 (14.0)	12.1 – 15.9	157 (12.2)	10.4–14.0
NA	6	0 (0)	–	1 (16.7)	0 – 46.5
Living environment					
Rural	1356	187 (13.8)	12.0 – 15.6	163 (12.0)	10.3–13.7
Urban	71	1 (1.4)	4.4 – 19.6	0 (0)	–
NA	6	0 (0)	–	1 (16.7)	14.9–18.4
Type of Hunting					
Birds	815	31 (3.8)	3.0–4.7	14 (1.7)	1.1–2.3
Wild mammals	612	157 (25.7)	23.7 – 27.8	148 (24.2)	22.2–26.2
NA	6	0 (0)	–	2 (33.3)	0 – 71.0
Tick infestation history					
No	714	80 (11.2)	8.9 – 13.5	73 (10.2)	8.0–12.4
Yes	712	107 (15.0)	12.4 – 17.6	89 (12.5)	10.1–14.9
NA	7	1 (14.3)	0 – 40.2	2 (28.6)	0.00–62.1
Treatment months					
1	53	8 (15.1)	5.5 – 24.7	6 (11.3)	2.8–19.8
2–6	825	105 (12.8)	10.5 – 15.1	94 (11.4)	9.2–13.6
>6	538	73 (13.6)	10.7 – 16.5	61 (11.4)	8.7–14.1
NA	17	2 (8.3)	0 – 21.4	3 (12.5)	3.2–28.2

hunting dog population; furthermore, a significant association with concurrent infections by vector-borne pathogens, recognizing *R. sanguineus* s.l. as tick vector, such as *B. vogeli* and *H. canis*, has been observed, as well as previously described by Aktas and Ozubek (2018). Wengi et al. (2008) reported that most haemoplasma positive dogs in Switzerland had a history of travelling to countries where *R. sanguineus* s.l. is endemic and, other authors showed a significant association with *R. sanguineus* s.l. presence in Turkey (Aktas and Ozubek, 2018). Similarly, our risk factor analysis also supports the hypothesis that canine haemoplasmas can be transmitted by the brown dog tick, that is the most represented Ixodidae species in southern Italy (Maurelli et al., 2018). However, another study failed to find a similar association, although it must be noted that these organisms can cause chronic bloodstream infections, due to past tick exposure (Barker et al., 2010). Also, under field conditions Aktas and Ozubek (2017) failed to demonstrate trans-stadial transmission of canine haemoplasmas species by *R. sanguineus* s.l.; only a dated experimental study documents *R. sanguineus* s.l. transmission of *Mhc* to dogs (Seneviratna et al., 1973). For these reasons, having only indirect evidence for vector competence, the role of the brown dog tick in the transmission epidemiology of *Mhc* and *CMhp* must be further investigated.

A variety of risk factors related to haemoplasma infection have been reported in previous surveys that considered different canine study populations. Our data suggest that outdoor lifestyle and, contact with wild fauna could be important factors influencing exposure to haemoplasma infections in dogs, potentially due to either frequent exposure to tick infestations or possible wounds acquired during hunting or fighting. Ravagnan et al. (2017) reported a negligible haemoplasma prevalence in owned candidate blood donor dogs, while Aktas and Ozubek (2018) showed a higher infection prevalence in shelter and free-roaming stray dogs compared to pet dogs, suggesting a protective role of the owner care (e.g. regular treatment with ectoparasiticide drugs). However, in our study no significant difference emerged on the effects of the anti-ectoparasite use in preventing haemoplasma infection of dogs. This finding can be explained by the great variety of ectoparasiticidal compounds, dosage criteria and administration scheme (compliance) administered by hunters. Moreover, the role of vectors other than ticks, such as fleas, cannot be excluded. In Patagonia *Mhc* was found in up to 40 % of fleas (*Pulex irritans*) collected on grey foxes (*Lycalopex griseus*), with a significant association between the presence of DNA in the hosts and their fleas, but not in ticks (*Amblyomma tigrinum*) simultaneously collected from the animals (Millàn et al., 2019). Haemotropic *Mycoplasma* phylotypes were also detected in fleas (*Synosternus cleopatrae*) collected from rodents (*Gerbillus andersoni*) in Israel (Cohen et al., 2015). However, no haemotropic mycoplasmas were found in fleas collected in a large-scale survey on 622 dogs in the UK (Abdullah et al., 2019).

In our hunting dog population, breed category and wild mammal hunting were significant risk factors for acquiring haemoplasma infections. Hounds, particularly when compared to other hunting breed, have a greater close contact with wild mammals. As reported by other

Table 2
Logistic regression results for the risk factor eff ;ect associated with Mhc and CMhp positivity for hunting dogs in southern Italy.

	Mhc			*CMhp*		
Variable	OR	P value	95 % CI	OR	P value	95 % CI
Province						
Salerno	3.72	< 0.0001	2.65 – 5.21	2.74	< 0.0001	1.94 – 3.86
Avellino	0.56	< 0.0001	0.40 – 0.79	0.78	0.17	0.55 – 1.10
Napoli	0.02	< 0.0001	0.00 – 0.16	0.01	< 0.0001	0.00 – 0.20
Breed category						
Sheperd	19.92	0.131	0.81 - 491.37	2.57	1.000	0.10 - 63.41
Terrier	1.47	0.645	0.32 - 6.89	0.77	1.000	0.01 - 6.07
Hound	5.26	< 0.0001	3.76 - 7.36	8.46	< 0.0001	5.69 - 12.56
Pointing	0.11	< 0.0001	0.08 - 0.17	0.06	< 0.0001	0.03 - 0.10
Mixed – Breed	3.38	< 0.0001	1.91 - 5.98	2.8	0.0024	1.52 - 5.16
Body size						
Small				0.70	1.000	0.16 - 3.00
Medium				0.69	0.05	0.48 - 0.99
Large				1.48	0.043	1.02 – 2.14
Years						
< 2				1.19	<0.0001	0.086 - 0.33
2–7				2.01	0.0005	1.33 – 3.05
>7				1.84	0.020	1.10 – 3.08
Gender						
Male	1.44	0.0228	1.05 – 1.97			
Female	0.69	0.0275	0.50 - 0.95			
Pack size						
<10	0.66	0.0306	0.46 - 0.94	0.71	0.08	0.48 – 1.04
>10	2.96	< 0.0001	1.99 – 4.40	1.61	0.03	1.06 – 2.44
Cohabitation with other animals						
No	0.37	0.0037	0.17 - 0.77	0.31	0.0031	0.13 - 0.73
Yes	2.82	0.0027	1.36 – 5.86	2.78	0.0058	1.27 – 6.05
Living environment						
Rural	12.58	0.0004	1.68 – 87.99	10.38	0.0014	1.43 – 75.21
Urban	0.08	0.0009	0.01- 0.65	0.05	0.0003	0.00 - 0.82
Type of hunting						
Birds	0.11	< 0.0001	0.07 - 0.17	0.10	< 0.0001	0.05 - 0.18
Wild mammals	8.73	< 0.0001	5.84 – 13.05	8.32	< 0.0001	4.91 – 14.10
Tick infestation history						
No	0.71	0.0347	0.52- 0.97			
Yes	1.40	0.0346	1.02 – 1.91			

authors, the close contact of hunting dogs with wild mammals or bush/woodland results in more frequent exposure to several tick-borne infections (Ebani et al., 2015; Piantedosi et al., 2017). In this regard, the questing behaviour used by some Ixodid ticks is well known; the ticks wait on vegetation for long periods and when they sense a host approaching, extend their front legs and cling to the host hair coat (Dantas-Torres, 2008). Although previously shown in domestic cats (Willi et al., 2010), the role of aggressive interactions in domestic dogs, and wild canids, as a haemoplasma transmission route is currently under discussion (Compton et al., 2012; Millàn et al., 2019). Recently, Dear et al. (2018) reported coinfection with haemoplasma (both *Mhc* and *CMhp*) and *Babesia conradae* in Greyhounds dogs with a history of coyote fighting, although only *B. conradae* infection was associated with hematological abnormalities.

In the current study, the risk factor analysis revealed a significantly higher prevalence in dogs living in Salerno province, which is known to have large wooded areas with high densities of wildlife (ENETwild et al., 2018). Conversely, living in the more urbanized province of Napoli, where most of the animals are used for bird hunting, proved to be a protective factor (only one dog positive to *Mhc*; 0.4 %).

Pack size more than 10 animals was another associated risk factor for canine haemoplasma infection, likely related to the group-housing which could increase risk of exposure to vector agents as well as potential direct haemoplasma transmission. Finally, adult age was significantly associated with infection by *CMhp*, probably due to a longer exposure to the organism, rather than an increased susceptibility to the infection in older dogs.

5. Conclusion

In conclusion, our data contribute to the current epidemiological understanding of canine haemotropic mycoplasma infection in Italy, although not all haemoplasma species that infect dogs were investigated. The present study confirms the circulation of *Mhc* and *CMhp* within the hunting dog population of southern Italy. Haemoplasma infected dogs did not exhibit clinical signs referable to the specific haemoplasma agent. For this reason, further studies are needed to determine the clinical relevance of hemotropic mycoplasma infection in this at-risk population and, to evaluate the relationship between hunting dogs and sympatric populations of wild animals in the epidemiology of *Mhc* and *CMhp*.

Finally, future studies are important to spread the knowledge of canine haemoplasma in veterinary community and in hunters in order to ensure the health of dogs, and given their possible zoonotic role, to enhance the safety of human beings.

Declaration of Competing Interest

The study was supported by the management committees of the hunting districts of Napoli (ATC NA), Avellino (ATC AV) and Salerno (ATC SA 1) provinces of Campania region, Italy. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper. MB, JB, PT and RC are employees of IDEXX Laboratories. Inc., Westbrook, Maine, USA.

Acknowledgements

The authors thanks Dr. Paolo Varuzza for technical assistance in creating the map.

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