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# Seroprevalence and associated risk factors for vector-borne pathogens in dogs from Egypt

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## Abstract

**Background:** Dogs play an important role as reservoirs of many zoonotic vector-borne pathogens worldwide, yet reports of canine vector-borne diseases (CVBDs) in Egypt are scarce.

**Methods:** Serum samples were collected from pet dogs ( $n = 500$ ) of the three most common breeds (German Shepherd, Rottweiler and Pit Bull) in five Governates of Cairo ( $n = 230$ ), Giza ( $n = 110$ ), Al-Qalyubia ( $n = 60$ ), Al-Gharbia ( $n = 60$ ) and Kafr El-Sheikh ( $n = 40$ ) with a hot desert climate. The presence of antibodies to *Anaplasma* spp. (*A. phagocytophilum*, *A. platys*), *Ehrlichia* spp. (*E. canis*, *E. chaffeensis*, *E. ewingii*), *Borrelia burgdorferi* (s.l.) and *Dirofilaria immitis* were assessed using IDEXX SNAP<sup>®</sup> 4Dx<sup>®</sup> ELISA tests. For each pathogen, risk factors (i.e. geographical area, keeping condition, sex, age, breed, tick infestation, weekly sanitation of dog enclosures and application of ectoparasiticides) were evaluated by logistic regression approach.

**Results:** In total, 18.2% ( $n = 91$ , 95% CI 15.1–21.8) of dogs scored seropositive for at least one pathogen, the most frequent being *Ehrlichia* spp. ( $n = 56$ ; 11.2%; 95% CI 8.7–14.3) followed by *Anaplasma* spp. ( $n = 33$ ; 6.6%, 95% CI 4.7–9.1), *Borrelia burgdorferi* (s.l.) ( $n = 9$ ; 1.8%, 95% CI 0.9–3.4) and *D. immitis* ( $n = 7$ ; 1.4%, 95% CI 0.9–2.9). In the tested population, 15.4% (95% CI 12.5–18.8) of dogs were exposed to a single pathogen while 2.4 (95% CI 1.4–4.2) and 0.4% (95% CI 0.1–1.4) were simultaneously exposed to two or three pathogens, respectively. Major risk factors associated with VBDs were living outdoors (*Anaplasma* spp.,  $P = 0.0001$ ; *Ehrlichia* spp.,  $P = 0.0001$ ), female sex (*Ehrlichia* spp.,  $P = 0.005$ ), German Shepherd breed (*Anaplasma* spp.,  $P = 0.04$ ; *Ehrlichia* spp.,  $P = 0.03$ ), tick infestation (*Anaplasma* spp.,  $P = 0.0001$ ; *Ehrlichia* spp.,  $P = 0.0001$ ; *B. burgdorferi* (s.l.),  $P = 0.003$ ; *D. immitis*,  $P = 0.02$ ), irregular sanitation (*Anaplasma* spp.,  $P = 0.0001$ ; *Ehrlichia* spp.,  $P = 0.0001$ ; *B. burgdorferi* (s.l.),  $P = 0.002$ ; *D. immitis*,  $P = 0.01$ ) and not using ectoparasiticides (*Anaplasma* spp.,  $P = 0.0001$ ; *Ehrlichia* spp.,  $P = 0.0001$ ; *B. burgdorferi* (s.l.),  $P = 0.007$ ).

**Conclusion:** To our knowledge, this is the first large-scale seroepidemiological study of CVBDs in Egypt. Considering that all of the detected pathogens are potentially zoonotic, effective ectoparasite control strategies, regular examination of pet dogs and successful chemoprophylaxis are advocated.

**Keywords:** *Anaplasma*, *Borrelia*, Canine vector-borne pathogens, *Dirofilaria*, Egypt, *Ehrlichia*, One-health, Zoonosis

## Background

Vector-borne diseases (VBDs) are of global importance especially in the case of zoonotic infections which pose a direct threat to animal and human health [1–3]. These pathogens are circulated in animal and human communities by arthropod vectors including ticks, mosquitoes, fleas and phlebotominae sand flies [4, 5]. Canine vector-borne diseases (CVBDs) of viral, bacterial and protozoal origin are often widespread in tropical and subtropical

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regions [6], including in the Middle East and North Africa (MENA), because of the favourable climatic conditions for the perpetuation of arthropod vectors and development of canine vector-borne pathogens (CVBPs) [7]. Among CVBPs, tick-borne *Ehrlichia* spp., *Anaplasma* spp., *Borrelia* spp. and mosquito-borne *Dirofilaria* spp. are of great importance for dogs [8].

Dogs are the main reservoir hosts for zoonotic gram-negative intracellular bacteria *Ehrlichia canis*, *E. ewingii* and *E. chaffeensis* [9, 10]. *Ehrlichia canis*, the causative agent of canine monocytic ehrlichiosis (CME), is transmitted by *Rhipicephalus sanguineus* (s.l.) and is prevalent in dog populations worldwide [11, 12]. The clinical outcome of ehrlichiosis varies from mild symptoms to fatal illness in the chronic phase depending on the strain, individual immune response and presence of concomitant infections [4]. Although dogs may show nonspecific signs (e.g., fever, depression, weakness, lethargy, anorexia, weight loss and a mucopurulent nasal discharge), asymptomatic *Ehrlichia* infection may also occur [13–15].

Human and animal infections with *Anaplasma* species are increasingly recognized as important, occasionally emerging and potentially fatal tick-transmitted diseases of humans and animals [16, 17]. Among six recognized species in the genus *Anaplasma*, *A. phagocytophilum*, the causative agent of granulocytic anaplasmosis, is diagnosed in a wide range of warm-blooded hosts including dogs, cats, horses, sheep, goats, cattle, camels and humans [18, 19]. *Anaplasma platys* is a common VBP of dogs in MENA [20, 21] and has occasionally been detected in humans [22–24]. Dogs usually are silent carriers of the infection [25], with clinical signs (e.g. fever, lethargy, anorexia and thrombocytopenia) sometimes described [26].

Among bacteria of the genus *Borrelia*, which affect different animal species including humans, *Borrelia burgdorferi* (s.l.) species complex causes Lyme disease, which is considered a major zoonosis for which many animals species (e.g. reptiles, rodents, wild ruminants) are reservoirs and *Ixodes* spp. tick the primary vector [27, 28]. In dogs, *B. burgdorferi* (s.l.) most often causes nonspecific signs (e.g., fever, apathy, lethargy, renal damage and lymphadenopathy) but also severe arthritis and neurological disorders [29]. However, in the endemic areas the majority of seropositive dogs do not present any clinical signs of the infection although they often remain persistently infected for approximately 1 year [30].

*Dirofilaria immitis* (Spirurida, Onchocercidae) is the causative agent of canine heartworm disease, which is transmitted through the bite of several mosquito species worldwide [31]. Dirofilariosis may also affect other mammals including humans, leading to the formation of pulmonary nodules, which may be often confounded with

pulmonary carcinoma [32]. Although most dogs infected by *D. immitis*—specially in endemic areas—are asymptomatic microfilaremic reservoirs, clinical signs depend on several factors, such as adult worm burden and localization [33]. The distribution of canine dirofilariosis in the MENA region, especially in North Africa, is not well known because of the paucity of epidemiological studies [31].

In Egypt, infection of dogs with *E. canis* [13, 34], *D. immitis* [35] and *B. burgdorferi* (s.l.) [36, 37] have been reported, in most cases based on small numbers of dogs and limited geographical areas. In this country *Rh. sanguineus* (s.l.) (brown dog tick), the competent vector of several tick-borne diseases [38, 39], has been prevalent in dog populations since ancient times [40–43]. DNA of *E. canis* and *A. phagocytophilum* has been detected in ticks attached to dogs [44, 45]. However, generally there are limited data on the occurrence of CVBDs in north African countries, e.g. Morocco [46], Algeria [47, 48] and Tunisia [49, 50].

The aim of the current study was to provide novel information on the seroprevalence and distribution of causative agents of monocytic ehrlichiosis, granulocytic anaplasmosis, Lyme disease and heartworm disease in dogs from five Governorates of Egypt and assess the risk factors associated with the infections.

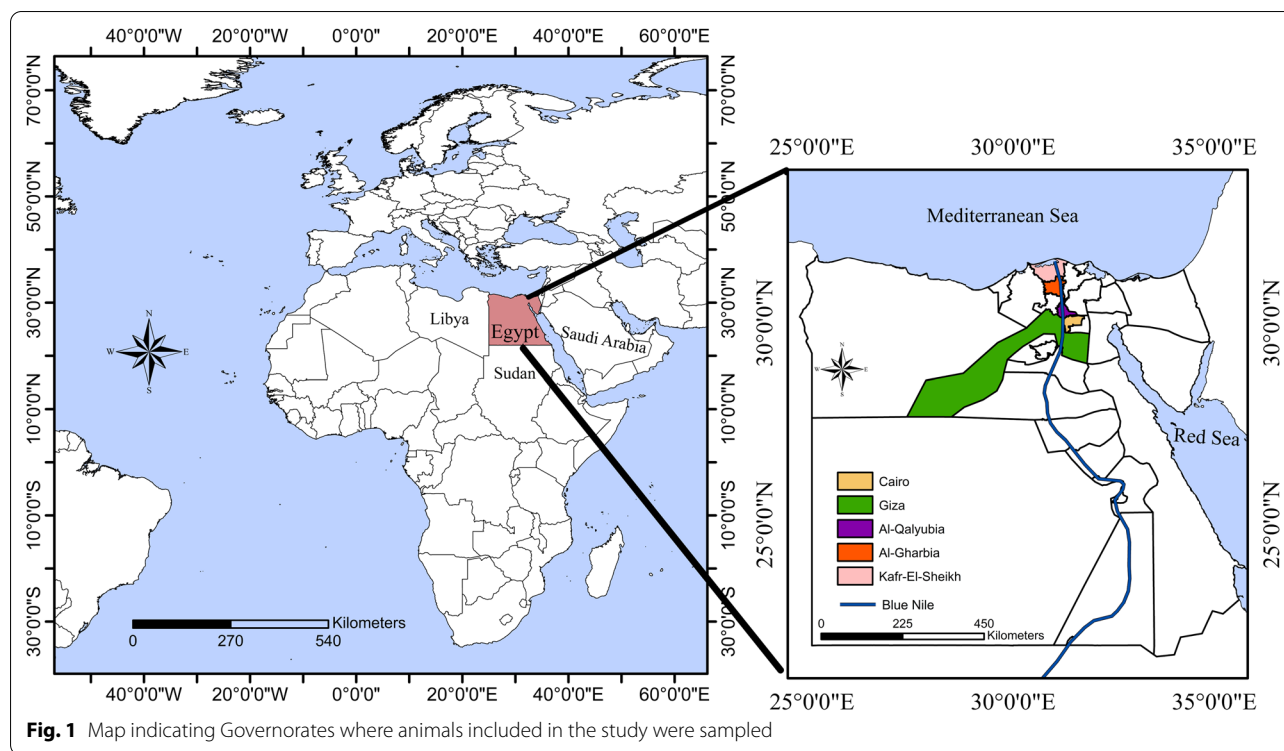
## Methods

### Study area

Egypt is a transcontinental country spanning the northeast corner of Africa and southwest corner of Asia. It is divided into 27 Governorates; the large regions of the Sahara desert, which constitute most of Egypt's territory, are sparsely inhabited. The investigation was conducted in Cairo (30.0444°N, 31.2357°E), Giza (30.0131°N, 31.2089°E), Al-Qalyubia (30.3292°N, 31.2168°E), Al-Gharbia (30.8754°N, 31.0335°E) and Kafr El-Shaikh (31.1107°N, 30.9388°E) (Fig. 1). These Governorates essentially have a hot desert climate, which is classified as BWh by the Köppen-Geiger system.

### Sample collection

During 2018 and 2019, blood samples (ca. 2 ml) were collected from the cephalic or saphenous veins of 500 dogs of the three most common breeds raised in Egypt, i.e. German Shepherd, Rottweiler and Pit Bull, admitted to veterinary clinics of five cities in different Governorates, namely Naser City in Cairo ( $n=230$ ), 6th of October in Giza ( $n=110$ ), Benha in Al-Qalyubia ( $n=60$ ), Tanta in Al-Gharbia ( $n=60$ ) and Kafr El-Sheikh in the governate of the same name ( $n=40$ ). Since other breeds of dogs are rarely kept as pets in Egypt, they were excluded from this study. Dogs were



**Fig. 1** Map indicating Governorates where animals included in the study were sampled

grouped according to age into four groups: < 1 year (G1); between 1 and 3 (G2); between 3 and 5 (G3); > 5 years (G4). Animal data (i.e. age, sex, breed, tick infestation, weekly sanitation of the dog enclosures and tri-monthly application of ectoparasiticides) were recorded.

**Serological examination**

Sera were separated by centrifugation of blood (1500×g for 10 min) and preserved at -20 °C until tested by IDEXX SNAP® 4Dx® (IDEXX Laboratories, Westbrook, ME, USA), which is a validated in-clinic ELISA test system. The kit simultaneously detects antibodies against immunodominant proteins of *E. canis*, *E. chaffeensis*, *E. ewignii* (peptides from p30 and p30-1 outer membrane proteins and p28 outer surface protein family), *A. phagocytophilum*, *A. platys* (peptide from the major surface protein p44/MSP2), *B. burgdorferi* (*s.l.*) (C6 peptide, derived from the IR6 region within the *Borrelia* membrane protein VlsE) [51] and *D. immitis* analyte derived from two antibodies (one for capture and the other for detection) specific to heartworm antigens [51–53]. The sensitivity and specificity of this kit are 93.2 and 99.2% for *A. phagocytophilum*, 89.2 and 99.2% for *A. platys*, 96.7 and 98.8% for *B. burgdorferi* (*s.l.*), 97.8 and 92.3% for *E. canis* and 98.9 and 99.3% for *D. immitis* [52–54].

**Statistical analysis**

Chi-square test and Fisher’s exact test were used to compare seropositivity to each pathogen, and the results were considered significant if  $P \leq 0.05$ . In particular,  $P$ -values were calculated with Fisher’s exact test only for variables below five (keeping condition, sex, weekly sanitation of the dog enclosures, presence of ticks on the dog body and tri-monthly application of ectoparasiticides) for *D. immitis* and *B. burgdorferi* (*s.l.*); all other  $P$ -values were calculated with Chi-square test. Univariable logistic regression analysis was used to evaluate the association of prevalence of each pathogen and variables of location (five Governorates), keeping condition (indoors or outdoors), sex (male or female), age (three groups), breed (three breeds), weekly sanitation of the dog enclosures, tick infestation and tri-monthly application of ectoparasiticides. Variables with  $P \leq 0.05$  in the univariable analyses were conducted to the multivariable models. To determine the risk probability of CBVDs, the odds ratio (OR) and confidence interval (CI) of significant variables were calculated using the multivariate logistic regression model. Multiple linear regression analysis was used to determine the possible multiple collinearities of different variables included in this study. The Hosmer-Lemeshow test was calculated to assess the goodness of fit for each model. Statistical analyses were carried out using SPSS software (ver. 24.0, IBM, USA).

## Results

Of 500 tested dogs, 91 (18.2%) scored seropositive for at least one pathogen, the most frequent being infection with *Ehrlichia* spp. ( $n=56$ ; 11.2%) followed by *Anaplasma* spp. ( $n=33$ ; 6.6%), *B. burgdorferi* (*s.l.*) ( $n=9$ ; 1.8%) and *D. immitis* ( $n=7$ ; 1.4%). In the tested population 15.4% of dogs were exposed to a single pathogen while 2.4% and 0.4% were simultaneously exposed to two or three pathogens, respectively (Table 1).

The risk of exposure to pathogens was significantly associated with keeping condition, sex, breed, tick infestation, weekly sanitation of the dog enclosures and tri-monthly application of ectoparasiticides (Table 2). In particular, the risk of infection with *Ehrlichia* spp., *Anaplasma* spp. and *B. burgdorferi* (*s.l.*) was significantly associated with living outdoors, and CME was most prevalent in female dogs. Regarding breed of dogs, German Shepherds showed higher seroprevalence of *Ehrlichia* spp., *Anaplasma* spp. and *B. burgdorferi* (*s.l.*). Importantly, a significantly higher chance of seropositivity to all CVBDs was observed in dogs that lived in enclosures that were not sanitized, did not undergo ectoparasiticide application and were infested with ticks (Table 3). No statistical association was found between CVBDs and other variables. Multicollinearity analysis showed strong correlations between seropositivity to *Anaplasma* spp. and *Ehrlichia* spp. and tick infestation, not receiving adequate hygienic care and ectoparasiticides where the variance inflation factor (VIF) was 15.665 and 25.117, respectively. However, such correlations were observed for seropositivity to *B. burgdorferi* (*s.l.*) and *D. immitis*, i.e. VIF was 1 and 1.082, respectively.

## Discussion

Data presented indicate that dog populations (i.e. 18.2%) in Egypt are exposed to CVBP, therefore posing threats to their own health and to people. This is largely due to the wide distribution of *Rh. sanguineus* (*s.l.*), the most common tick species infesting dogs in Egypt and a vector of canine ehrlichiosis and anaplasmosis [40–42]. Though little information is available about the prevalence of CVBDs in the MENA region, dogs often act as reservoirs of VBP with prevalence of 18.8% in Qatar [55], 24.5% in Saudi Arabia [24], 38.1% in Iraq [21], 46.9% in Iran [20], 69.7% in Algeria [48], 73% in Israel [56] and 83.8% in Morocco [46]. In Egypt CVBDs were mostly observed in urban Governorates (i.e. Giza, Cairo and Al-Qalyubia) where keeping pet animals is more common, also indicating that dogs and people in these regions are at higher risk of acquiring VBDs of canine origin.

The most prevalent pathogen diagnosed in this study was *Ehrlichia* spp. An *E. canis* seroprevalence of 41% was reported in dogs from Cairo and Alexandria [34] where DNA of *E. canis* was also detected in *Rh. sanguineus* (*s.l.*) ticks collected on dogs [45]. In addition, the presence of *E. canis* is supported by the wide distribution in Egypt of the brown dog tick *R. sanguineus* (*s.l.*) [40], which is the recognized vector for this species [57]. Although the employed test could detect exposure to *E. canis*, *E. chaffeensis* and *E. ewingii*, in a previous study only *E. canis*, but not *E. ewingii* or *E. chaffeensis*, was molecularly diagnosed in blood of 39 seropositive dogs from Cairo, Giza and Al-Qalyubia [58]. Nonetheless, since *E. ewingii* and *E. chaffeensis* have been diagnosed in dogs, ticks and human patients from different countries in the African continent, e.g. Cameroon, Mali, Uganda and South Africa [59–61],

**Table 1** Number and percentage of dogs ( $n=500$ ) seropositive to *Ehrlichia* spp., *Anapalsma* spp., *B. burgdorferi* (*s.l.*) and *D. immitis*

Pathogen	No. positive	Prevalence (%)	95% CI
Exposure to one pathogen			
<i>Ehrlichia</i> spp.	44	8.8	6.5–11.7
<i>Anapalsma</i> spp.	24	4.8	3.1–7.1
<i>B. burgdorferi</i> ( <i>s.l.</i> )	5	1	0.3–2.4
<i>D. immitis</i>	4	0.8	0.2–2.1
Exposure to two pathogens			
<i>Ehrlichia</i> spp. + <i>Anapalsma</i> spp.	5	1	0.3–2.4
<i>Ehrlichia</i> spp. + <i>B. burgdorferi</i> ( <i>s.l.</i> )	3	0.6	0.2–1.7
<i>Ehrlichia</i> spp. + <i>D. immitis</i>	2	0.4	0.1–1.4
<i>D. immitis</i> + <i>Anapalsma</i> spp.	2	0.4	0.1–1.4
Exposure to three pathogens			
<i>Ehrlichia</i> spp. + <i>Anapalsma</i> spp. + <i>D. immitis</i>	1	0.2	0.04–1.1
<i>Ehrlichia</i> spp. + <i>Anapalsma</i> spp. + <i>B. burgdorferi</i> ( <i>s.l.</i> )	1	0.2	0.04–1.1

**Table 2** Risk factors associated with seroprevalence rates of VBDs among dogs in Egypt with single and mixed infection (n = 91) according to different variables

Variable	No. <i>Ehrlichia</i> spp.			No. <i>Anaplasma</i> spp.			No. <i>Borrelia burgdorferi</i> (s.l.)			No. <i>Dirofilaria immitis</i>		
	Number (%)	95% CI <sup>a</sup>	Chi square df <sup>b</sup> P-value	Number (%)	95% CI	Chi square df P-value	Number (%)	95% CI	Chi square df P-value	Number (%)	95% CI	Chi square df P-value
Governate												
Cairo	230	24 (10.4)	7.1–15	15 (6.5)	3.9–10.5	χ <sup>2</sup> = 6.091 df = 4 P = 0.2	5 (2.2)	0.9–4.9	χ <sup>2</sup> = 2.813 df = 4 P = 0.6	4 (1.7)	0.6–4.3	χ <sup>2</sup> = 1.782 df = 4 P = 0.7
Giza	110	19 (17.2)	11.3–25.4	12 (10.9)	6.3–18.1		2 (1.8)	0.5–6.3		2 (1.8)	0.5–6.3	
Al-Qalyubia	60	6 (10)	4.6–20.1	2 (3.3)	0.9–11.3		2 (3.3)	0.9–11.3		1 (1.6)	0.3–8.8	
Al-Gharbia	60	4 (6.6)	2.6–15.9	3 (5)	1.7–13.7		0	0–6		0	0–6	
Kafr El-Sheikh	40	3 (7.5)	2.5–19.8	1 (2.5)	0.4–12.8		0	0–8.7		0	0–8.7	
Keeping condition												
Indoor	310	12 (3.8)	2.2–6.6	9 (2.9)	1.5–5.4	χ <sup>2</sup> = 44.060 df = 1 P = 0.0001	2 (0.6)	0.2–2.3	χ <sup>2</sup> = 6.155 df = 1 P = 0.03	2 (0.6)	0.1–2.3	χ <sup>2</sup> = 2.071 df = 1 P = 0.1
Outdoor	190	44 (23.2)	17.7–29.6	24 (12.6)	8.6–18.1		7 (3.6)	1.7–7.4		5 (2.6)	1.1–6.1	
Sex												
Male	230	16 (6.9)	4.1–11	11 (4.7)	2.6–8.3	χ <sup>2</sup> = 7.712 df = 1 P = 0.005	2 (0.8)	0.2–3.1	χ <sup>2</sup> = 2.086 df = 1 P = 0.2	2 (0.8)	0.2–3.1	χ <sup>2</sup> = 0.868 df = 1 P = 0.3
Female	270	40 (14.8)	11.1–19.5	22 (8.2)	5.4–12		7 (2.5)	1.2–5.2		5 (1.8)	0.7–4.2	
Age												
< 1 year	30	3 (10)	3.4–25.6	2 (6.6)	1.8–21.3	χ <sup>2</sup> = 7.525 df = 3 P = 0.06	1 (3.3)	0.5–16.6	χ <sup>2</sup> = 2.992 df = 3 P = 0.4	0	0–11.3	χ <sup>2</sup> = 1.525 df = 3 P = 0.6
1–3 years	210	16 (7.6)	4.7–12	11 (5.2)	2.9–9.1		2 (0.9)	0.2–3.4		2 (0.9)	0.2–3.4	
3–5 years	180	22 (12.2)	8.2–17.8	15 (8.3)	5.1–13.3		3 (1.6)	0.5–4.7		3 (1.6)	0.5–4.7	
> 5 years	80	15 (18.7)	11.7–28.6	5 (6.2)	2.7–13.8		3 (3.7)	1.2–10.4		2 (2.5)	0.6–8.6	
Breed												
German Shepherd	260	38 (14.6)	10.8–19.4	24 (9.2)	6.2–13.3	χ <sup>2</sup> = 6.870 df = 2 P = 0.03	6 (2.3)	1.1–4.9	χ <sup>2</sup> = 1.161 df = 2 P = 0.5	4 (1.5)	0.6–3.8	χ <sup>2</sup> = 0.550 df = 2 P = 0.7
Rottweiler	110	10 (9.1)	5–15.9	5 (4.5)	1.9–10.2		2 (1.8)	0.5–6.3		2 (1.8)	0.5–6.3	
Pit Bull	130	8 (6.2)	3.1–11.6	4 (3.1)	1.2–7.6		1 (0.7)	0.1–4.2		1 (0.7)	0.1–4.2	
Season												
Spring	190	26 (13.6)	9.5–19.3	12 (6.3)	3.6–10.7	χ <sup>2</sup> = 4.416 df = 3 P = 0.2	4 (2.1)	0.8–5.2	χ <sup>2</sup> = 1.400 df = 3 P = 0.7	3 (2.1)	0.8–5.2	χ <sup>2</sup> = 2.955 df = 3 P = 0.4
Summer	175	21 (12)	7.9–17.6	16 (9.1)	5.7–14.3		4 (2.2)	0.8–5.7		4 (2.2)	0.8–5.7	
Autumn	90	7 (7.7)	3.8–15.2	4 (4.4)	1.7–10.8		1 (1.1)	0.2–6		0	0.0–5.1	
Winter	45	2 (4.4)	1.2–14.8	1 (2.2)	0.3–11.5		0	0.0–9.8		0	0.0–9.8	
Weekly sanitation of the dog enclosures												
Yes	370	23 (6.2)	4.2–9.2	10 (2.7)	1.4–4.9	χ <sup>2</sup> = 35.346 df = 1 P = 0.0001	2 (0.5)	0.1–1.9	χ <sup>2</sup> = 12.771 df = 1 P = 0.002	2 (0.5)	0.1–1.9	χ <sup>2</sup> = 7.615 df = 1 P = 0.02
No	130	33 (25.3)	18.6–33.4	23 (18.5)	12.7–26		7 (5.4)	2.6–10.6		5 (3.8)	1.6–8.6	

**Table 2** (continued)

Variable	<i>Ehrlichia</i> spp.			<i>Anaplasma</i> spp.			<i>Borrelia burgdorferi</i> (s.l.)			<i>Dirofilaria immitis</i>			
	No.	Number (%)	95% CI <sup>a</sup>	Chi square df <sup>b</sup>	P-value	Number (%)	95% CI	Chi square df	P-value	Number (%)	95% CI	Chi square df	P-value
Presence of tick on the dog body													
Yes	140	48 (34.3)	26.9–42.5	$\chi^2 = 104.196$ df=1	<b>P=0.0001</b>	22 (15.7)	10.6–22.6	$\chi^2 = 26.203$ df=1	<b>P=0.0001</b>	7 (5)	2.4–9.9	$\chi^2 = 11.264$ df=1	<b>P=0.003</b>
No	360	8 (2.2)	1.1–4.3			11 (3.1)	1.7–5.3			2 (0.5)	0.12		
Tri-monthly application of ectoparasiticides													
Yes	370	24 (6.4)	4.4–9.4	$\chi^2 = 31.790$ df=1	<b>P=0.0001</b>	11 (2.9)	1.6–5.2	$\chi^2 = 30.370$ df=1	<b>P=0.0001</b>	2 (0.5)	0.1–1.9	$\chi^2 = 8.604$ df=1	<b>P=0.007</b>
No	130	32 (24.6)	18–32.6			22 (16.9)	11.4–24.2			7 (5.4)	2.6–10.6		
Total	500	56 (11.2)	8.7–14.3			33 (6.6)	4.7–9.1			9 (1.8)	0.9–3.3		

P-values were calculated with Fisher's exact test only for variables below five (keeping condition, sex, weekly sanitation of the dog enclosures, presence of tick on the dog body and tri-monthly application of ectoparasiticides) for *D. immitis* and *B. burgdorferi* (s.l.); all other P-values were calculated with Chi-square test

Significant variables (P-values ≤ 0.05) are marked in bold

<sup>a</sup> Confidence interval

<sup>b</sup> Degrees of freedom

**Table 3** Multivariate logistic regression analysis of risk factors associated with seroprevalence rates of VBDs in dogs in Egypt with single and mixed infection ( $n=91$ ) according to different variables

Pathogen	Factor	$\beta^a$	SE <sup>b</sup>	Odds ratio	95% Confidence interval	$P$
<i>Ehrlichia</i> spp.	Keeping condition					
	Outdoor	2.013	0.314	7.5	3.8–14.6	0.0001*
	Sex					
	Female	0.844	0.311	2.3	1.2–4.2	0.007†
	Breed					
	Pit Bull (constant)	–	–	–	–	–
	German Shepherd	0.959	0.405	2.6	1.2–5.7	0.02‡
	Rottweiler	0.422	0.493	1.5	0.6–4	0.3
	Weekly sanitation of the dog enclosures					
	No	1.636	0.295	5.1	2.8–9.1	0.000†
<i>Anaplasma</i> spp.	Presence of tick on the dog body					
	Yes	3.143	0.399	22.9	10.4–50.2	0.000†
	Tri-monthly application of ectoparasiticides					
	No	1.549	0.293	4.7	2.6–8.4	0.000†
	Keeping condition					
	Outdoor	1.576	0.403	4.8	2.1–10.6	0.00†
	Breed					
	German Shepherd	1.117	0.553	3.1	1.1–9.1	0.04‡
	Rottweiler	0.405	0.684	1.5	0.4–5.7	0.5
	Weekly sanitation of the dog enclosures					
No	2.046	0.394	7.7	3.5–16.7	0.000†	
<i>B. burgdorferi</i> (s.l.)	Presence of tick on the dog body					
	Yes	1.778	0.384	5.9	2.7–12.5	0.000†
	Tri-monthly application of ectoparasiticides					
	No	1.894	0.385	6.6	3.1–14.1	0.000†
	Keeping condition					
	Outdoor	1.773	0.807	5.8	1.2–28.6	0.02‡
	Weekly sanitation of the dog enclosures					
	No	2.349	0.809	10.5	2.1–51.1	0.004‡
	Presence of tick on the dog body					
	Yes	2.243	0.808	9.4	1.9–45.9	0.006‡
<i>D. immitis</i>	Tri-monthly application of ectoparasiticides					
	No	2.439	0.809	10.5	2.1–51.1	0.004‡
	Weekly sanitation of the dog enclosures					
	No	1.966	0.843	7.4	1.4–38.4	0.02‡
	Presence of tick on the dog body					
Yes	1.673	0.843	6.6	1.3–34.5	0.02‡	
Tri-monthly application of ectoparasiticides						
No	1.966	0.843	7.4	1.4–38.4	0.02‡	

$\beta$ : Wald statistic; SE: standard error; 95% CI: 95% confidence interval; OR: odds ratio

\*These parameters are statistically significant

further investigations should be carried out to characterize the species of *Ehrlichia* infecting dogs in Egypt.

To the best of our knowledge this is the first report of sero-reaction to *A. phagocytophilum*/*A. platys* in

dogs from Egypt (6.6%). It seems that *A. phagocytophilum* infection of dogs is not prevalent in some regions of MENA, also considering that previous studies from Saudi Arabia, Qatar, Iraq and five regions of Iran failed

to detect the infection [20, 21, 55, 62]. However, both *A. phagocytophilum* and *A. platys* were detected in *Rh. sanguineus* (*s.l.*) blood-feeding on dogs in Cairo and Giza [44, 45] and *A. phagocytophilum* in blood of five human patients in the Nile Delta [63].

The detection of nine dogs (1.8%) seropositive to *B. burgdorferi* (*s.l.*) suggests that the pathogen circulates in Egypt as indicated by previous studies where prevalence ranged from 23% [36] to 71.4% [37]. In addition, some tick species, e.g. *Rh. sanguineus* (*s.l.*), *Rh. annulatus*, *Hyalomma excavatum*, *Hy. dromedarii*, *Amblyomma lepidum* and *Ornithodoros savignyi*, scored molecularly positive for DNA of *B. burgdorferi* (*s.l.*) [36, 64]. The circulation of *B. burgdorferi* (*s.l.*) in Egypt has also been demonstrated [36, 63, 65, 66]. In addition, tick-borne relapsing fever (TBRF) caused by *Borrelia persica*, *Borrelia microti*, *Borrelia latyschewii* and *Borrelia baltazardi* is also endemic in MENA [67] including Egypt where relapsing fever *Borrelia* spp. were detected in *Ornithodoros savignyi* ticks and sera of camel, sheep, goat, cattle and buffalo [68, 69]. In particular, *B. persica*, which is transmitted by *Ornithodoros tholozani*, can infect dogs and has been reported from Egypt, Israel, Iran, Pakistan and former USSR Asian republics including Uzbekistan [70, 71]. Considering that yet available commercial point-of-care diagnostic kits such as SNAP® 4Dx® employed in this study do not detect TBRF in dogs, complementary tests for dogs living in the endemic areas are recommended.

The detection of seven dogs (1.4%) from Giza, Cairo and Al-Qalyubia seropositive to *D. immitis* corroborates an older report of microfilariae of *D. immitis* in blood smears from 8/19 imported German Shepherd dogs in Assiut Governorate, Upper Egypt [35]. In particular, antibodies to *D. immitis* were already detected from cats of Giza with prevalence of 3.4% [72]. While human cases with *D. repens* have been frequently reported in Egypt [73, 74], *D. immitis* was described once in a patient [75]. Data for canine dirofilariasis by *D. immitis* in North Africa are limited to few reports from Tunisia [49, 76], Algeria [77, 78] and Morocco [46]. It has been suspected that *D. immitis* is absent from some Middle Eastern countries such as Israel where *D. repens* is present [79]. In contrast, weighted prevalence of *D. immitis* infection in dog populations of Turkey and Iran was estimated to be 11.32% and 11.45%, respectively [80]. However, in a recent study in five geographical regions of Iran testing 354 dogs, no microfilaremic dog was found [20].

According to our findings, dogs that were living outdoors had a higher risk of being seropositive to *Ehrlichia* spp., *Anaplasma* spp. and *B. burgdorferi* (*s.l.*) probably as an effect of the higher chances of being exposed to bites of the brown dog tick *Rh. sanguineus* (*s.l.*) and other tick

species that are competent vectors of these pathogens [5]. Furthermore, as expected, dogs that did not receive adequate hygienic care and antiparasitic treatments were more likely to be affected by CVBDs. Regular application of ectoparasiticides with potent anti-feeding and fast killing effects, repellents, insect growth regulators and juvenile hormone analogues combined with environmental treatment to reduce the number of adult and juvenile ticks are key control measures in managing CVBDs [81]. Ectoparasiticides, available in several formulations, such as pour-on, spot-on, shampoos, sprays and collars, have long-lasting effects [82] and are highly recommended to dog owners to prevent CVBDs.

In this study, seropositivity to *Ehrlichia* spp. was more frequent in female dogs. In contrast, some studies have found higher seropositivity to CVBDs in males due to behavioural characteristics that cause greater exposure to vectors than females [83, 84]. No sex-related correlation was recorded for other tested pathogens in this study.

German Shepherds showed higher seroprevalences of *Ehrlichia* spp. and *Anaplasma* spp. Although all breeds are prone to CVBDs, CME has been reported most frequently in the German Shepherds [85, 86]. German Shepherd dogs and Siberian Huskies are predisposed to developing more severe clinical signs of ehrlichiosis; therefore, these breeds have a worse prognosis [14]. An experimental study showed that the cell-mediated immune response to a challenge with *E. canis* was reduced in German Shepherds compared to Beagle dogs [87]. Examination of other dog breeds in Egypt though are very rare, and “*baladi*” stray dogs will shed light on the true occurrence of CVBDs in the country.

As a limitation of the employed commercial kit, PCR-positive/antibody-negative and antibody-positive/PCR-negative dogs have been reported [88]. The first case could represent early infections before the development of antibody responses; the second case may represent cases of past infections that may have been treated or spontaneously resolved. Furthermore, this commercial kit detects both IgM and IgG antibodies against *Ehrlichia* spp., *Anaplasma* spp. and *B. burgdorferi* (*s.l.*). Although chronic long-term bacteremia is characteristic for some rickettsial agents, long-term persistence of IgG could occur, not reflecting the real “infection” status in some animals. Hence, seropositivity values must be interpreted as current infection with or previous exposure to the pathogens under assessment. Finally, the prevalence of *D. immitis* infection in Egypt should be carefully considered as an alarm bell for the introduction of a parasite not common in Egypt but which is expanding its range of distribution in southern regions of the Mediterranean Basin such as southern Italy [89].



## Conclusion

The present study is the first comprehensive study for CVBD pathogens that has been conducted in Egypt to our knowledge and confirms the presence of *Ehrlichia* spp., *Anaplasma* spp., *B. burgdorferi* (s.l.) and *D. immitis* in dogs of different regions. The connection between these VBPs and their arthropod vectors in Egypt remains largely unknown and warrants further investigation. Considering that all of the detected pathogens are known zoonotic pathogens, effective ectoparasite control strategies, regular examination of pet dogs and successful chemoprophylaxis are advocated.

## Abbreviations

CVBPs: Canine vector-borne pathogens; CVBDs: Canine vector-borne diseases; OR: Odds ratio; MENA: Middle East and North Africa; DNA: Deoxyribonucleic acid; CME: Canine monocytic ehrlichiosis; TBRF: Tick-borne relapsing fever; CI: Confidence interval; ELISA: Enzyme-linked immunosorbent assay; s.l.: Sensu lato; VIP: Variance inflation factor; IR6: Invariable region 6; VlsE: Vmp-like sequence; df: Degrees of freedom.

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## Authors' contributions

ASe conceived the study and performed field work. ASe, ADA and ASA performed laboratory work and analysed data. ASA and ASe wrote the first draft of the manuscript. DO reviewed the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional files.

## Declarations

### Ethics approval and consent to participate

Blood samples for this study were approved by the Ethical Research Committee, Benha University, Egypt (Approval no.: BUFVTM045) and collected after owners' consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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## References

1. Yuasa Y, Hsu T-H, Chou C-C, Huang C-C, Huang W-C, Chang C-C. The comparison of spatial variation and risk factors between mosquito-borne and tick-borne diseases: seroepidemiology of *Ehrlichia canis*, *Anaplasma* species, and *Dirofilaria immitis* in dogs. *Comp Immunol Microbiol Infect Dis*. 2012;35(6):599–606.
2. de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, et al. Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. *Front Cell Infect Microbiol*. 2017;7:114.
3. Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: a one health perspective. *Trends Parasitol*. 2012;28(10):437–46.
4. Otranto D, Dantas-Torres F, Breitschwerdt EB. Managing canine vector-borne diseases of zoonotic concern: part one. *Trends Parasitol*. 2009;25(4):157–63.
5. Angelou A, Gelasakis AI, Verde N, Pantchev N, Schaper R, Chandrashekar R, et al. Prevalence and risk factors for selected canine vector-borne diseases in Greece. *Parasit Vectors*. 2019;12(1):283.
6. Nguyen V, Colella V, Greco G, Fang F, Nurcahyo W, Hadi U, et al. Molecular detection of pathogens in ticks and fleas collected from companion dogs and cats in East and Southeast Asia. *Parasit Vectors*. 2020;13:420.
7. Kilpatrick AM, Randolph SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet*. 2012;380(9857):1946–55.
8. Günaydin E, Turut N, Akin B, Koç Ö, Ütük AE. A serosurvey on some canine vector-borne zoonoses (*Anaplasma* spp., *Ehrlichia* spp., *Borrelia burgdorferi*, *Dirofilaria immitis* and *Leishmania* spp.) in Osmaniye. *Atatürk Üniv Vet Bilim Derg*. 2019;14(2):151–8.
9. Breitschwerdt EB, Hegarty BC, Hancock SI. Sequential evaluation of dogs naturally infected with *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia equi*, *Ehrlichia ewingii*, or *Bartonella vinsonii*. *J Clin Microbiol*. 1998;36(9):2645–51.
10. Ismail N, Bloch KC, McBride JW. Human ehrlichiosis and anaplasmosis. *Clin Lab Med*. 2010;30(1):261–92.
11. Cardoso L, Mendão C, de Carvalho LM. Prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* sensu lato, *Anaplasma* spp. and *Leishmania infantum* in apparently healthy and CVBD-suspect dogs in Portugal—a national serological study. *Parasit Vectors*. 2012;5(1):62.
12. Lee S, Lee H, Park J-W, Yoon S-S, Seo H-J, Noh J, et al. Prevalence of antibodies against *Anaplasma* spp., *Borrelia burgdorferi* sensu lato, *Babesia gibsoni*, and *Ehrlichia* spp. in dogs in the Republic of Korea. *Ticks Tick Borne Dis*. 2020;11:101412.
13. Salem NY, Rakha GH, Baraka TA. Naturally occurring ehrlichiosis in Egyptian dogs. *Iran J Vet Res*. 2014;15(1):54–7.
14. Sainz Á, Roura X, Miró G, Estrada-Peña A, Kohn B, Harrus S, et al. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasit Vectors*. 2015;8(1):75.
15. Harrus S, Aroch I, Lavy E, Bark H. Clinical manifestations of infectious canine cyclic thrombocytopenia. *Vet Rec*. 1997;141(10):247–50.
16. Mendoza-Roldan J, Santhakumari Manoj R, Latrofa M, Iatta R, Annoscia G, Lovreglio P, et al. Role of reptiles and associated arthropods in the epidemiology of rickettsioses: a one health paradigm. *PLOS Negl Trop Dis*. 2021;15(2):e0009090.
17. Sazmand A, Harl J, Eigner B, Hodžić A, Beck R, Hekmatimoghaddam S, et al. Vector-borne bacteria in blood of camels in Iran: new data and literature review. *Comp Immunol Microbiol Infect Dis*. 2019;65:48–53.
18. Alanazi AD, Nguyen VL, Alyousif MS, Manoj RR, Alouffi AS, Ridolfi D, et al. Ticks and associated pathogens in camels (*Camelus dromedarius*) from Riyadh Province, Saudi Arabia. *Parasit Vectors*. 2020;13:110.
19. Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol*. 2013;3:31.
20. Iatta R, Sazmand A, Nguyen V-L, Nemati F, Bahiraei Z, Mazhar Ayaz M, et al. Vector-borne pathogens in dogs of different regions of Iran and Pakistan. *Parasitol Res*. 2021. <https://doi.org/10.1007/s00436-020-06992-x>.
21. Otranto D, Iatta R, Baneth G, Cavalera MA, Bianco A, Parisi A, et al. High prevalence of vector-borne pathogens in domestic and wild carnivores in Iraq. *Acta Trop*. 2019;197.
22. Breitschwerdt EB, Hegarty BC, Qurollo BA, Saito TB, Maggi RG, Blanton LS, et al. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*,

- and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. *Parasit Vectors*. 2014;7(1):298.
23. Arraga-Alvarado CM, Qurollo BA, Parra OC, Berrueta MA, Hegarty BC, Breitschwerdt EB. Molecular evidence of *Anaplasma platys* infection in two women from Venezuela. *Am J Trop Med Hyg*. 2014;91(6):1161–5.
  24. Alanazi AD, Alouffi AS, Alyousif MS, Alshahrani MY, Abdullah HHAM, Abdel-Shafy S, et al. Molecular survey of vector-borne pathogens of dogs and cats in two regions of Saudi Arabia. *Pathogens*. 2021;10(1):25.
  25. Kohn B, Silaghi C, Galke D, Arndt G, Pfister K. Infections with *Anaplasma phagocytophilum* in dogs in Germany. *Res Vet Sci*. 2011;91(1):71–6.
  26. Granick JL, Armstrong PJ, Bender JB. *Anaplasma phagocytophilum* infection in dogs: 34 cases (2000–2007). *J Am Vet Med Assoc*. 2009;234(12):1559–65.
  27. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet*. 2012;379(9814):461–73.
  28. Mendoza-Roldan JA, Colella V, Lia RP, Nguyen VL, Barros-Battesti DM, Iatta R, et al. *Borrelia burgdorferi* (sensu lato) in ectoparasites and reptiles in southern Italy. *Parasit Vectors*. 2019;12(1):35.
  29. Adaszek Ł, Gatellet M, Mazurek Ł, Dębiak P, Skrzypczak M, Winiarczyk S. Myocarditis secondary to *Borrelia* infection in a dog: a case report. *Ann Parasitol*. 2020;66(2):255–7.
  30. Goossens H, Van Den Bogaard A, Nohlmans M. Dogs as sentinels for human Lyme borreliosis in The Netherlands. *J Clin Microbiol*. 2001;39(3):844–8.
  31. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, et al. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. *Clin Microbiol Rev*. 2012;25(3):507–44.
  32. Dantas-Torres F, Otranto D. Overview on *Dirofilaria immitis* in the Americas, with notes on other filarial worms infecting dogs. *Vet Parasitol*. 2020;282:
  33. Panarese R, Iatta R, Latrofa MS, Zatelli A, Ćupina AI, Montarsi F, et al. Hyperendemic *Dirofilaria immitis* infection in a sheltered dog population: an expanding threat in the Mediterranean region. *Int J Parasitol*. 2020;50(8):555–9.
  34. Botros B, Elmolla M, Salib A, Calamaio C, Dasch G, Arthur R. Canine ehrlichiosis in Egypt: sero-epidemiological survey. *Onderstepoort J Vet Res*. 1995;62:41–3.
  35. Abd El-Rahim I. Canine dirofilariasis among imported dogs in upper Egypt. *Ass Vet Med J*. 1998;40:121–32.
  36. Elhelw RA, El-Enbaawy MI, Samir A. Lyme borreliosis: a neglected zoonosis in Egypt. *Acta Trop*. 2014;140:188–92.
  37. Samir A, Fahmy A, Hatem EM, Orabi A. Occurrence of canine borreliosis in Egyptian dogs: a public health threat. *Transl Biomed*. 2015;6(29):1–4.
  38. Latrofa MS, Dantas-Torres F, Giannelli A, Otranto D. Molecular detection of tick-borne pathogens in *Rhipicephalus sanguineus* group ticks. *Ticks Tick borne Dis*. 2014;5(6):943–6.
  39. Dantas-Torres F. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Vet Parasitol*. 2008;152(3–4):173–85.
  40. Aziz ARA, Abou Laila MR, Bazh EK, Sultan K. Epidemiological study on brown dog tick *Rhipicephalus sanguineus* at Sadat District, Egypt. *PSM Vet Res*. 2017;2(1):1–5.
  41. El-Gawady HM. Studies on ectoparasites of stray dogs in Ismailia city. *Egypt Vet Med Soc Parasitol J*. 2015;11(1):115–22.
  42. Amin OM, Madbouly MH. Distribution and seasonal dynamics of a tick, a louse fly, and a louse infesting dogs in the Nile Valley and Delta of Egypt. *J Med Entomol*. 1973;10(3):295–8.
  43. Otranto D, Huchet J-B, Giannelli A, Callou C, Dantas-Torres F. The enigma of the dog mummy from Ancient Egypt and the origin of '*Rhipicephalus sanguineus*'. *Parasit Vectors*. 2014;7:2.
  44. Ghafar MW, Amer SA. Prevalence and first molecular characterization of *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt. *J Adv Res*. 2012;3(2):189–94.
  45. Nasr A, El Hariri M, Ghafar MW. Detection of *Anaplasma platys* and *Ehrlichia canis* in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt; a public health concern. *Vet Med J Giza*. 2020;66:1–9.
  46. Khatat SE, Khallaayoune K, Errafyk N, Van Gool F, Duchateau L, Daminet S, et al. Detection of *Anaplasma* spp. and *Ehrlichia* spp. antibodies, and *Dirofilaria immitis* antigens in dogs from seven locations of Morocco. *Vet Parasitol*. 2017;239:86–9.
  47. Dahmani M, Loudahi A, Mediannikov O, Fenollar F, Raoult D, Davoust B. Molecular detection of *Anaplasma platys* and *Ehrlichia canis* in dogs from Kabylie, Algeria. *Ticks Tick Borne Dis*. 2015;6(2):198–203.
  48. Azzag N, Petit E, Gandoïn C, Bouillin C, Ghalmi F, Haddad N, et al. Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers. *Comp Immunol Microbiol Infect Dis*. 2015;38:1–7.
  49. Rjeibi M, Rouatbi M, Mabrouk M, Tabib I, Rekiq M, Gharbi M. Molecular study of *Dirofilaria immitis* and *Dirofilaria repens* in dogs from Tunisia. *Transbound Emerg Dis*. 2017;64(5):1505–9.
  50. M'ghirbi Y, Ghorbel A, Amouri M, Nebaoui A, Haddad S, Bouattour A. Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. *Parasitol Res*. 2009;104(4):767–74.
  51. Pantchev N, Schnyder M, Vrhovec MG, Schaper R, Tsachev I. Current surveys of the seroprevalence of *Borrelia burgdorferi*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Leishmania infantum*, *Babesia canis*, *Angiostrongylus vasorum* and *Dirofilaria immitis* in dogs in Bulgaria. *Parasitol Res*. 2015;114(1):117–30.
  52. Chandrashekar R, Mainville CA, Beall MJ, O'Connor T, Eberts MD, Aleman AR, et al. Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* and *Dirofilaria immitis* antigen in dogs. *J Am Vet Med Assoc*. 2010;71(12):1443–50.
  53. Stillman BA, Monn M, Liu J, Thatcher B, Foster P, Andrews B, et al. Performance of a commercially available in-clinic ELISA for detection of antibodies against *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* and *Dirofilaria immitis* antigen in dogs. *J Am Vet Med Assoc*. 2014;245(1):80–6.
  54. Pantchev N, Pluta S, Huisinga E, Nather S, Scheufelen M, Vrhovec MG, et al. Tick-borne diseases (borreliosis, anaplasmosis, babesiosis) in German and Austrian dogs: status quo and review of distribution, transmission, clinical findings, diagnostics and prophylaxis. *Parasitol Res*. 2015;114(5):519–54.
  55. Alho AM, Lima C, Latrofa MS, Colella V, Ravagnan S, Capelli G, et al. Molecular detection of vector-borne pathogens in dogs and cats from Qatar. *Parasit Vectors*. 2017;10:298.
  56. Baneth G, Breitschwerdt EB, Hegarty BC, Pappalardo B, Ryan J. A survey of tick-borne bacteria and protozoa in naturally exposed dogs from Israel. *Vet Parasitol*. 1998;74(2–4):133–42.
  57. Groves M, Dennis G, Amyx H, Huksoll D. Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). *Am J Vet Res*. 1975;36(7):937–40.
  58. Selim A, Abdelhady A, Alahadeb J. Prevalence and first molecular characterization of *Ehrlichia canis* in Egyptian dogs. *Pak Vet J*. 2020. <https://doi.org/10.29261/pakvetj/2020.061>.
  59. Ndip LM, Ndip RN, Esemu SN, Walker DH, McBride JW. Predominance of *Ehrlichia chaffeensis* in *Rhipicephalus sanguineus* ticks from kennel-confined dogs in Limbe, Cameroon. *Exp Appl Acarol*. 2010;50(2):163.
  60. Uhaa IJ, Maclean JD, Greene CR, Fishbein DB. A case of human ehrlichiosis acquired in Mali: clinical and laboratory findings. *Am J Trop Med Hyg*. 1992;46(2):161–4.
  61. Proboste T, Kalema-Zikusoka G, Altet L, Solano-Gallego L, De Mera IGF, Chirife AD, et al. Infection and exposure to vector-borne pathogens in rural dogs and their ticks, Uganda. *Parasit Vectors*. 2015;8:306.
  62. Chandra S, Smith K, Alanazi AD, Alyousif MS, Emery D, Ślapeta J. *Rhipicephalus sanguineus* sensu lato from dogs and dromedary camels in Riyadh, Saudi Arabia: low prevalence of vector-borne pathogens in dogs detected using multiplexed tandem PCR panel. *Fol Parasitol*. 2019;66:007.
  63. Ghafar MW, Eltablawy NA. Molecular survey of five tick-borne pathogens (*Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato and *Babesia microti*) in Egyptian farmers. *Glob Vet*. 2011;7(3):249–55.
  64. Adham FK, Abd-El-Samie E, Gabre RM, El Hussein H. Detection of tick blood parasites in Egypt using PCR assay II-*Borrelia burgdorferi* sensu lato. *J Egypt Soc Parasitol*. 2010;40(3):553–64.
  65. Haberberger RL Jr, Constantine NT, Schwan TG, Woody JN. Lyme disease agent in Egypt? *Trans R Soc Trop Med Hyg*. 1989;83(4):556.
  66. Hammouda NA, Hegazy IH, El-Sawy E. ELISA screening for Lyme disease in children with chronic arthritis. *J Egypt Soc Parasitol*. 1995;25(2):525–33.
  67. Naddaf SR, Ghazinezhad B, Sedaghat MM, Asl HM, Cutler SJ. Tick-borne relapsing fever in southern Iran, 2011–2013. *Emerg Infect Dis*. 2015;21(6):1078.

68. Helmy N. Seasonal abundance of *Ornithodoros (O.) savignyi* and prevalence of infection with *Borrelia* spirochetes in Egypt. *J Egypt Soc Parasitol.* 2000;30(2):607–19.
69. Shanbaky NM, Helmy N. First record of natural infection with *Borrelia* in *ornithodoros (Ornithodoros) savignyi*. Reservoir potential and specificity of the tick to *Borrelia*. *J Egypt Soc Parasitol.* 2000;30(3):765–80.
70. Baneth G, Nachum-Biala Y, Halperin T, Hershko Y, Kleinerman G, Anug Y, et al. *Borrelia persica* infection in dogs and cats: clinical manifestations, clinicopathological findings and genetic characterization. *Parasit Vectors.* 2016;9(1):244.
71. Shirani D, Rakhshanpoor A, Cutler SJ, Ghazinezhad B, Naddaf SR. A case of canine borreliosis in Iran caused by *Borrelia persica*. *Ticks Tick Borne Dis.* 2016;7(3):424–6.
72. Al-Kappany Y, Lappin M, Kwok O, Abu-Elwafa S, Hilali M, Dubey J. Seroprevalence of *Toxoplasma gondii* and concurrent *Bartonella* spp., feline immunodeficiency virus, feline leukemia virus, and *Dirofilaria immitis* infections in Egyptian cats. *J Parasitol.* 2011;97(2):256–8.
73. Awadalla H, Bayoumi DM, Ibrahim IR. The first case report of suspected human dirofilariasis in the eyelid of a patient from Alexandria. *J Egypt Soc Parasitol.* 1998;28:941–3.
74. Abdel-Rahman S, Mahmoud A, Galal L, Gustinelli A, Pampiglione S. Three new cases of human infection with *Dirofilaria repens*, one pulmonary and two subcutaneous, in the Egyptian governorate of Assiut. *Ann Trop Med Parasitol.* 2008;102(6):499–507.
75. Elnazer M. Human ectopic infection with *Dirofilaria immitis* (Leydig, 1865) representing first record from Egypt. *Egypt Med J.* 1994;11:435–8.
76. Chabchoub A, Landolsi F, Selmi C. Serological survey and clinical study of dirofilariasis with *Dirofilaria immitis* carried out in working dogs in Tunis region (Tunisia). *Rev Méd Vét.* 2003;154(11):673–8.
77. Meriem-Hind B-M, Mohamed M. Prevalence of canine *Dirofilaria immitis* infection in the city of Algiers, Algeria. *Afr J Agric Res.* 2009;4(10):1097–100.
78. Tahir D, Damene H, Davoust B, Parola P. First molecular detection of *Dirofilaria immitis* (Spirurida: Onchocercidae) infection in dogs from Northern Algeria. *Comp Immunol Microbiol Infect Dis.* 2017;51:66–8.
79. Capelli G, Genchi C, Baneth G, Bourdeau P, Brianti E, Cardoso L, et al. Recent advances on *Dirofilaria repens* in dogs and humans in Europe. *Parasit Vectors.* 2018;11(1):663.
80. Anvari D, Narouei E, Daryani A, Sarvi S, Moosazadeh M, Hezarjaribi HZ, et al. The global status of *Dirofilaria immitis* in dogs: a systematic review and meta-analysis based on published articles. *Res Vet Sci.* 2020;131:104–16.
81. Dantas-Torres F, Otranto D. Dogs, cats, parasites, and humans in Brazil: opening the black box. *Parasit Vectors.* 2014;7(1):22.
82. Otranto D. Arthropod-borne pathogens of dogs and cats: from pathways and times of transmission to disease control. *Vet Parasitol.* 2018;251:68–77.
83. Costa LM Jr, Rembeck K, Ribeiro MFB, Beelitz P, Pfister K, Passos LMF. Seroprevalence and risk indicators for canine ehrlichiosis in three rural areas of Brazil. *Vet J.* 2007;174(3):673–6.
84. Cortes S, Vaz Y, Neves R, Maia C, Cardoso L, Campino L. Risk factors for canine leishmaniasis in an endemic Mediterranean region. *Vet Parasitol.* 2012;189(2–4):189–96.
85. Huxsoll DL, Hildebrandt PK, Nims RM, Amyx HL, Ferguson JA. Epizootiology of tropical canine pancytopenia. *J Wildl Dis.* 1970;6(4):220–5.
86. Jafari S, Gaur S, Hashemi A. Prevalence of *Ehrlichia canis* in dog population of Shiraz, Fars province of Iran. *J Appl Anim Res.* 1997;11(1):19–23.
87. Nyindo M, Huxsoll D, Ristic M, Kakoma I, Brown J, Carson C, et al. Cell-mediated and humoral immune responses of German shepherd dogs and beagles to experimental infection with *Ehrlichia canis*. *Am J Vet Res.* 1980;41(2):250–4.
88. Wong SS, Teng JL, Poon RW, Choi GK, Chan K-H, Yeung ML, et al. Comparative evaluation of a point-of-care immunochromatographic test SNAP 4Dx with molecular detection tests for vector-borne canine pathogens in Hong Kong. *Vector Borne Zoonotic Dis.* 2011;11(9):1269–77.
89. Mendoza-Roldan J, Benelli G, Panarese R, Iatta R, Furlanello T, Beugnet F, Zatelli A, Otranto D. *Leishmania infantum* and *Dirofilaria immitis* infections in Italy, 2009–2019: changing distribution patterns. *Parasit Vectors.* 2020;13(1):193.

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