Research Article

Detection of Anaplasmosis and Ehrlichiosis in Blood of Owned Dogs in Alexandria, Northern Egypt

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Abstract | Anaplasmosis and ehrlichiosis are important tick-borne diseases in dogs. The current study aimed to evaluate the occurrence of anaplasmosis and ehrlichiosis in owned dogs in Alexandria, northern Egypt. Accordingly, a total of 70 dogs, admitted to private clinics for veterinary care, were surveyed and blood specimens were taken for both Giemsa-stained microscopic examination and molecular detection using conventional PCR. The present study revealed that 8.57% (6/70) dogs were infected with *Anaplasma/Ehrlichia* spp. Based on the examination of Giemsa-stained blood smears, *Anaplasma platys* was found in blood platelets of 4 (5.71%) dogs and *Ehrlichia canis* was revealed inside macrophages of 2 (2.86%) dogs. No mixed infections were recovered. All infected dogs had an infestation with *Rhipicephalus sanguineus* ticks. Variable clinical signs were detected in the surveyed dogs, often loss of appetite, loss of body weight and lethargy. Among risk factors, it has been found that dogs aged less than 2 years were the highest infected as well as male dogs were more susceptible to the infection. cPCR-based molecular investigation targeting the 16sRNA gene revealed that 20.0% (14/70) of dogs were positive for *Anaplasma/Ehrlichia* species with a specific amplicon size of 345 bp. Expectedly, the use of PCR-based molecular tool was more accurate than the conventional microscopic examination. Further literature based on both traditional and molecular tools targeting another hemoparasites infecting dogs together with an accurate identification of the associated tick vector are urgently needed to achieve a successful control of those pathogens.

Keywords | *Anaplasma platys*, *Ehrlichia canis*, Dogs, Microscopy, PCR, Egypt

INTRODUCTION

Family Anaplasmataceae (Order Rickettsiales) are obligate intracellular pathogens capable of infecting mostly mononuclear phagocytes of dogs and cats. Members of such family replicate within a vacuole derived from the host cell membrane (Dumler et al., 2001; Ybanez et al., 2012). In the beginning of 21ˢᵗ century, based on phylogenetic analyses, those species were reclassified, and the family included the genera *Anaplasma*, *Ehrlichia*, and *Neorickettsia* with various members were renamed, like

Ehrlichia platys which is renamed to *Anaplasma platys* (Allison and Little, 2013).

Canine vector-borne diseases constitute a major veterinary health obstacle in Mediterranean districts (Hamel et al., 2012). Among the causative agent of those, both *Anaplasma platys* and *Ehrlichia canis* are potential pathogens of veterinary concern (Ebani, 2019). *Anaplasma platys* was first detected in 1978 in Florida, USA, and it was initially known as *Ehrlichia platys*. It replicates inside blood platelets causing a symptomatic condition in infected dogs,

however, life-threatening pancytopenia, (canine infectious cyclic thrombocytopenia, CICT), via damage of platelets due to severe anaplasmosis, has been reported (Huang et al., 2005; Aguirre et al., 2006; Ramos et al., 2014).

Ehrlichia canis was first detected in 1935 in Algeria and it has a great affinity to monocytes and macrophages with acute clinical symptoms mostly fever and anemia in infected dogs (Aguirre et al., 2004) causing tropical canine pancytopenia, recently, canine monocytic ehrlichiosis (CME) in both tropical and subtropical countries (Harrus and Waner, 2011; Ramos et al., 2014; Sainz et al., 2015; Malik et al., 2018). *E. canis* often infect dogs, although literature denoted the infection in cats (Ebani et al., 2017). Moreover, human infections associated with clinical signs similar to those of canine monocystic ehrlichiosis (CME) developed revealing it a potentially zoonotic pathogen (Perez et al., 2006).

The genus *Rhipicephalus* (Acari: Ixodidae) includes several species with the *R. sanguineus*, the brown dog tick, group is frequently predominant in urban and rural areas of tropical and subtropical districts (Horak et al., 2013; Latrofa et al., 2014; Aktas, 2014; Malik et al., 2018) and most of them of veterinary and medical importance to dogs and humans (Dantas-Torres, 2008; Dantas-Torres et al., 2012; Latrofa et al., 2014). So far, the taxonomy of *R. sanguineus* comprises a complex issue with at least 4 taxa, particularly *R. sanguineus* sensu lato, have been recognized (Dantas-Torres et al., 2013a). Both *A. platys* and *E. canis* are evidently transmitted via the brown dog tick, *R. sanguineus* (Yabsley et al., 2008; Ramos et al., 2014).

In the past few decades, the PCR-based molecular detection of tick-borne pathogens in dogs has been increased. The present study aimed to detect the occurrence of *Anaplasma platys* and *Ehrlichia canis* in owned dogs in northern Egypt by the microscopic examination of Giemsa-stained blood films as well as molecularly by the use of conventional PCR in owned dogs' blood.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING

A total of 70 owned dogs (47 males and 23 females) were admitted, for a routine examination, treatment or vaccination, to private clinics in Alexandria, northern Egypt (coordinates: 31° 12′ 0″ N, 29° 55′ 0″ E). Dog breeds were German Shepherd (average weight 27-36 kg), Husky (average weight 17-25 kg), Golden Retriever (average weight 28-35 kg), Rottweiler (average weight 35-52 kg), Cane Corso (average weight 37-46 kg) and Mastiff (average weight 39-54 kg). Among the surveyed dogs, 42 animals were ticks-infested, 7 dogs were apparently ticks free but with a previous history of infestation and 21 dogs were not infested with ticks. According to the age, dogs are categorized into 3 sections; less than 2 years (40 dogs), 2-5 years (24 dogs) and more than 5 years (6 dogs). The whole blood (*n*=70) from the cephalic vein was taken by the use of 5 ml-syringe and added to tubes containing ethylene diamine tetraacetic acid (EDTA).

MICROSCOPIC EXAMINATION

Thin blood smears were fixed in methanol (99.5%) for 5 min and stained for 30 min in Giemsa solution diluted with 5% buffer. Slides were carefully examined for the occurrence of members of Anaplasmataceae. The smears were recorded as negative if no pathogens were detected in more than 50 oil-immersion microscopic fields (El-Dakhly et al., 2018).

DNA EXTRACTION AND PCR AMPLIFICATION

The remaining blood was used for PCR. DNA was extracted from 200 µl of EDTA-anticoagulated blood samples from dogs using the purification kit QIAamp DNA blood mini kit® (QIAGEN GmbH, Hilden, Germany). PCR amplification for the detection *Anaplasma* spp./ *Ehrlichia* spp. and *Babesia* spp./ *Hepatozoon* spp. was conducted using 25 µl-volume reactions: 12.5 master mix, 1 µl forward primer, 1 µl reverse primer, 7.5 µl DNA and RNA free water and 3 µl DNA from each sample. The used primers and cycling conditions were listed in Table 1.

Table 1: Primers and cycling conditions of the tested pathogens in examined dogs.

Parasite	Primer	Target gene	Product size (bp)	Cyclic conditions	References
Anaplasma spp./ *Ehrlichia* spp.	EHR16SD: 5′-GGTACCYACAGAA-GAAGTCC- 3′ EHR16SR: 5′-TAGCACTCATCGT-TTACA GC-3′	16S rRNA	345	95°C-120 sec, 94°C-60 sec, 54°C-30 sec (x40), 72°C-30 sec, 72°C- 300 sec	Martin et al. (2005)
Babesia spp./ *Hepatozoon* spp.	B18S-F 5′-TGG TTG ATC CTG CCA GTA-3′ B18S-R 5′-CTT CTC CTT CCT TTA AGT TGA-3′	18S rRNA	1665	94 °C- 30 sec,94 °C- 30 sec,56 °C- 2min (x30),72 °C- 2 min,72 °C- 5 min.	Sasaki et al. (2007); Spolidorio et al. (2009)

RESULTS AND DISCUSSION

The clinical examination of dogs revealed that 42 (60.0%) out of 70 were infested with ticks, *Rhipicephalus sanguineus*. Among the tick-infested dogs, 27.14% (19/70) had loss of appetite, loss of body weight and lethargy. The remaining dogs (40.0%; 28/70) were submitted for the routine examination and vaccination.

The microscopic examination of 70 Giemsa-stained blood smears (in less than 50 microscopic fields) revealed 6 (8.57%) dogs were infected with *Anaplasma/Ehrlichia* spp. The intensity of infection ranged from 1 to 3 protozoan/field. Among those, 5.71% (4/70) were only positive for *Anaplasma platys*. The pathogen was recognized as inclusion bodies in the blood platelets of infected dogs (Figure 1). Moreover, *Ehrlichia canis* was found in 2 (2.86%) dogs. It appeared as multiple rounded-shaped and relatively large-sized morulae inside macrophages (Figure 2). Dogs infected with both pathogens were infested with the associated brown dog tick, *Rhipicephalus sanguineus*, with the intensity of infestation ranged from 1-16 ticks (Figure 3). Based on the microscopic examination, no mixed infections were detected.
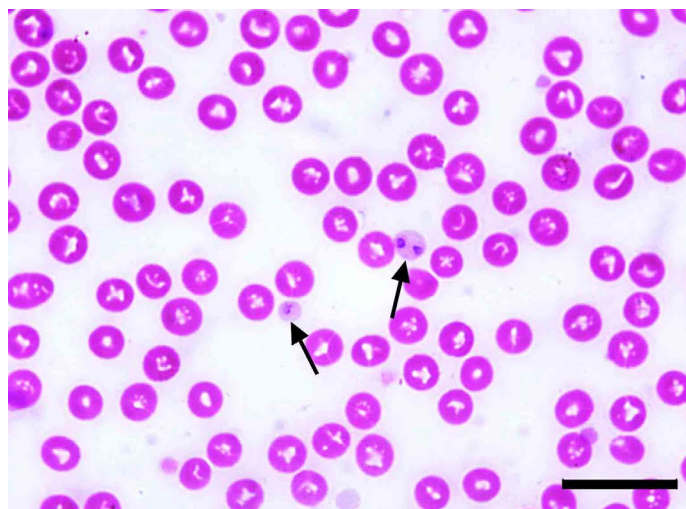

Figure 2: *Ehrlichia canis* inside a macrophage in Giemsa-stained blood smear of an infected dog. Note the multiple, relatively large-sized and round-shaped morulae intracellularly (*arrows*). Scale bar= 20 um.

Figure 3: The adult brown tick, *Rhipicephalus sanguineus*, infesting the surveyed dogs. a) A dog ear infested with the tick vector. b) Doral view of the adult tick. c) Anteroventral view of the adult tick. d) Posterventral view of the adult tick. Scale bar= 0.5 mm.

Figure 1: *Anaplasma platys* inclusion bodies in blood platelets (*arrows*) of Giemsa-stained blood smear of an infected dog. Scale bar= 20 um.

Surprisingly, neither *Babesia* nor *Hepatozoon* species could be detected in the examined samples. The examination of more than 50 fields was almost doubtful with a great tendency for all samples to be *Babesia/Hepatozoon* free. Therefore, a specific primer for both was used to absolutely exclude the absence of those pathogens.

Concerning the age, it has been found that dogs aged less than 2 years were highly infected, while elderly dogs were the lowest (Table 2). The current study revealed that male dogs were highly infected than females (Table 3).
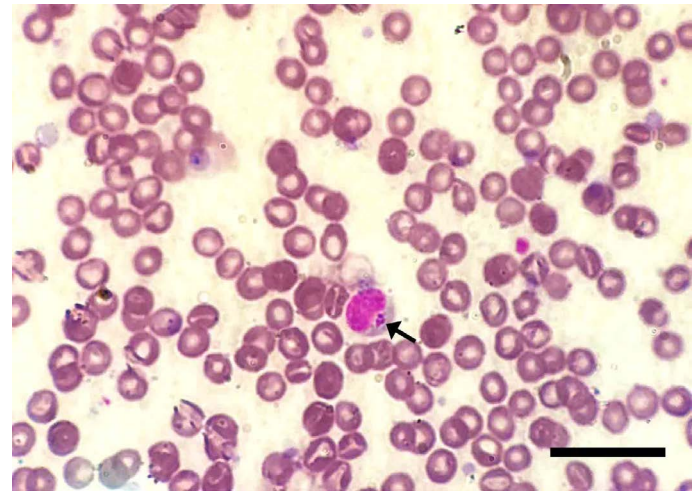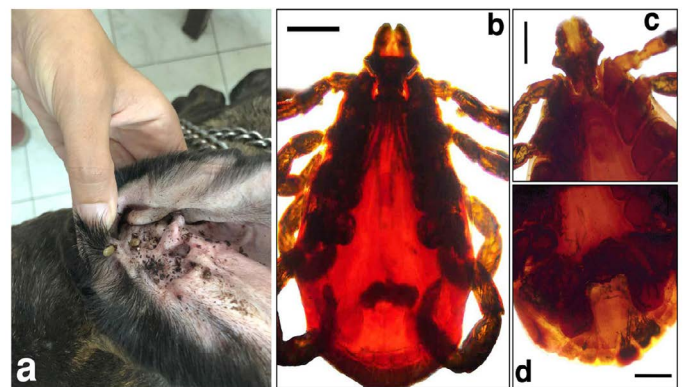
Table 2: The infection of *Anaplasma/Ehrlichia* species relative to the age of surveyed dogs.

Anaplasma/Ehrlichia infected dogs (%)	Examined dogs	Age
9 (12.86%)	40	< 2 years
3 (4.28%)	24	2-5 years
2 (2.86%)	6	>5 years
14 (20.0%)	70	Total

Table 3: The infection of *Anaplasma/Ehrlichia* species relative to the gender of surveyed dogs.

Anaplasma/Ehrlichia infected dogs (%)	Examined dogs	Gender
9 (12.86%)	47	Male
5 (7.14%)	23	Female
14 (20.0%)	70	Total

The molecular investigation of the surveyed samples showed that 20.0% (14/70) of dogs were positive for *Anaplasma/ Ehrlichia* species, using a common primer for both pathogens, with a specific amplicon size of 345 bp (Figure 4). *Babesia/Hepatozoon* species could not be detected among the examined samples. Expectedly, the use of PCR-based molecular tool was more accurate than the conventional direct microscopy.

Figure 4: PCR amplification of DNA extracted from blood of surveyed dogs targeting the 16S rRNA gene using a primer specific for *Anaplasma/Ehrlichia* spp. *Lane* M: a ladder of 100 bp. *Lanes* 1,2 showed control negative and control positive respectively. *Lanes* 3–12 showed 10 blood samples positive for *Anaplasma/Ehrlichia* spp. with a product of an amplicon size 345 bp.

The occurrence of canine vector-borne pathogens is a global concern for both pets' clinics and public health (Maggi et al., 2013; Ribeiro et al., 2017). The infection with Anaplasmataceae often based on the pathogen species, immune status of the host as well as the co-infections with other pathogens (Dahmani et al., 2019). The current work revealed that *A. platys* and *E. canis* were found in blood of examined dogs by the use of both microscopic examination and PCR, in Alexandria, northern Egypt. More or less similarly, Botros et al. (1995) examined both military kenneled and private owned dogs and they revealed that the overall ehrlichiosis was 33.0% using florescence antibody test. A recent study in Giza, Egypt showed that, based on PCR, percentages were 1.32% and 1.98% for both *A. platys* and *E. canis*, respectively. The discrepancy in percentages of infection might be due to the topography of both surveyed provinces as well as it might be attributed to the source of extracted DNA used in PCR (DNA is extracted from ticks in the later study and from blood in the current study) (Nasr et al., 2020). Meanwhile, Selim et al. (2021) recovered that the prevalence of *E. canis* using PCR-based molecular identification was 9.70%. Moreover, another report in Egypt demonstrated clinical signs of canine ehrlichiosis in 85 dogs without the detection of the pathogen by the microscopic examination in Giemsa-stained smears (Salem et el., 2014). Furthermore, Ghafar and Amer (2012) found that the prevalence of *A. phagocytophilum* in ticks associated with dogs was 13.7%

based on PCR targeting 16S rRNA gene.

In other Arabian countries, like Qatar, based on PCR findings, infection rates for *Anaplasma platys* and *Ehrlichia canis* were 1.6% and 3.1%, respectively. The lower percentage than the current investigation, 20.0%, might be referred to more veterinary care. Zaid et al. (2019) recorded that the infection rate of *A. platys* and *E. canis* in dogs blood samples in 9 districts of Palestine was 10% and 1.5%, respectively.
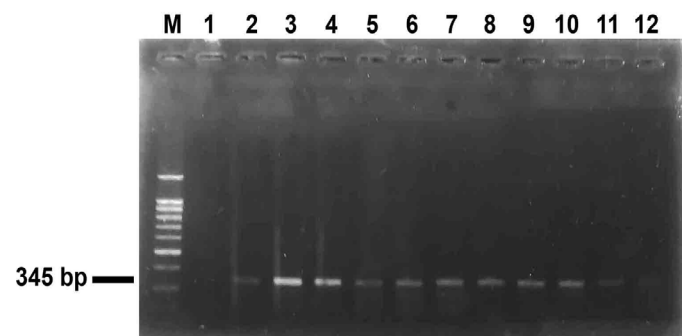
Closely related to our findings, Dahmani et al. (2019) reported infection rates of 15.6% and 18.8% for *A. platys* and *E. canis*, respectively using molecular investigation in Keur Momar Sarr area, Senegal. The later is an African country probably assuming more or less similar climate changes and topographical nature providing the existence of the same tick vector, *Rhipicephalus sanguineus*. In Nigeria, Adamu et al. (2014) recovered that either *A. platys* or *E. canis* was found in 7.0% of the surveyed dogs using PCR and RLB (Reverse Line Blot) assays. In Harare, Zimbabwe, Dhliwayo et al. (2019) detected that the overall prevalence of canine ehrlichiosis was 75.2% using ELISA. The higher infection rate might be attributed to the possible false results which could be achieved.

Out of the African continent, variable prevalences of anaplasmosis/ehrlichiosis were obtained. Among them, In Grenada, Yabsley et al. (2008) noted that the percentages of *A. platys* and *E. canis* infections were 19.2% and 24.7%, respectively. Kelly et al. (2013) reported that prevalences of *A. platys* and *E. canis* using 16S rRNA-based PCR in dogs St. Kitts in the Caribbean were 11.0% and 27.0%, respectively. In Brazil, Tanikawa et al. (2013) found that the infection rate with *E. canis* was 69.4% and 3.7% based on serological assay and RT-PCR, respectively. Ramos et al. (2014) detected that 18 (52.9%) dogs were positive for *A. platys* in southern Italy. Almazan et al. (2016) revealed that 31.0% and 10.0% of examined dogs were positive to *A. platys* and *E. canis*, respectively using 16S rRNA amplification-based PCR in north-central Mexico. Ybañez et al. (2018) detected that the prevalence of *Anaplasma/ Ehrlichia* infection in dogs using peripheral blood smear examination and PCR was 10.0% in Cebu, Philippines. Ebani (2019) found that 16.18% of samples were positive for *E. canis* and 3.31% for *A. phagocytophilum* using an indirect immunofluorescent assay in Italy. Amazingly, Zheng et al. (2017) reported neither *Anaplasma platys* nor *Ehrlichia canis* infection in surveyed dogs in 10 provinces of China. The current study reported that the use of advanced tools like 16S RNA-dependant PCR is more accurate than Giemsa-stained direct microscopy for detecting either *A. platys* or *E. canis* as a result of artifacts of the staining procedure as well as the misdiagnosis of the pathogen morulae intracellularly (Kelly et al., 2013; Salem et al., 2014; Almazan et al., 2016).

Currently, the percentage of anaplasmosis/ehrlichiosis in dogs aged less than one year were the highest, while dogs of more than 5 years were the lowest with male dogs were highly infected than females. Authors suggested that this issue might be due to a less veterinary care in the beginning of life together with the abundance of the tick vector, *R. sanguineus*, in the locality where dogs are sampled (Botros et al., 1995). Unlike these findings, Tanikawa et al. (2013) and Selim et al. (2020) reported that elderly dogs were more susceptible to the infections with both *A. platys* and *E. canis*. They referred this finding to the fact that aged dogs are more exposed to the infection together with the persistence of the tick vector along their life. Meanwhile, Ribeiro et al. (2017), Malik et al. (2018) and Dhliwayo et al. (2019) found that age, breed and sex have no effect on anaplasmosis/ehrlichiosis. Interestingly, Díaz-Sánchez et al. (2020) reported that the existence of ticks is not correlated with the severity of the infection.

The brown dog tick, *R. sanguineus*, is the main vector for the transmission of *Anaplasma platys* and *Ehrlichia canis* in tropical and subtropical areas. In Egypt, the detection of those pathogens in dogs and their associated ticks in various localities of the Mediterranean area is potentially correlated to the climate changes and ecosystem suggesting the continuation of the dog-tick life cycle (Pennisi et al., 2012; Dantas-Torres et al., 2013b; Latrofa et al., 2014; Almazán et al., 2016). It is worthy to mention that ticks seemed to be pathogens-free due to being that dogs are apparently healthy, as in previously treated dogs, therefore, the circulating antigens could not be detected (Almazán et al., 2016).

In the authors opinion, the overall discrepancies in the prevalence's of anaplasmosis/ehrlichiosis might be referred to a variety of factors; the lack of veterinary care and awareness of people towards dog populations particularly in poorly developed districts, climatic changes and topographical characters in several localities in such a way to allow the wide spread and extent of tick infestation in dogs, diagnostic tools used as well as the close contact between dogs and humans providing the potential role of those pathogens as zoonotic vector-borne pathogens (Galay et al., 2018). Due to the highly possible morbidity and mortality, biologists and veterinarians have to be exclusively alert to provide a well veterinarian care as well as the elimination of associated ticks that will reduce the spread of those tick-borne pathogens. Thus, the use of both direct microscopy and PCR-based molecular tools are highly recommended.

ACKNOWLEDGMENTS

NOVELTY STATEMENT

Authors declare that the manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

AUTHOR'S CONTRIBUTION

Khaled Mohamed El-Dakhly examined dog samples, collected raw data, identified pathogens and wrote the manuscript. Waleed M. Arafa examined samples, stained blood films and did PCR, Lilian N Mahrous contributed in editing, Amany S. Aboshinaf examined blood samples and did PCR. Magdy M. Tawfik collected samples, stained blood smears, did PCR.

COMPLIANCE WITH ETHICAL STANDARDS

Dog blood samples were collected in clinics according to well practice by veterinarians and according to the Egyptian regulations with the agreement of the owners.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

• Adamu M, Troskie M, Oshadu DO, Malatji DP, Penzhorn BL, Matjila PT (2014). Occurrence of tick-transmitted pathogens in dogs in Jos, Plateau State, Nigeria. Parasit. Vectors, 7: 119 http://www.parasitesandvectors.com/content/7/1/119. https://doi.org/10.1186/1756-3305-7-119

• Aguirre E, Sainz A, Dunner S, Amusategui I, Lopez L, Rodriguez-Franco F, Luaces I, Cortes O, Tesouro MA (2004). First isolation and molecular characterization of *Ehrlichia canis* in Spain. Vet. Parasitol., 125: 365-372. https://doi.org/10.1016/j.vetpar.2004.08.007

• Aguirre E, Tesouro MA, Ruiz L, Amusategui I, Sainz A (2006). Genetic characterization of *Anaplasma* (*Ehrlichia*) *platys* in dogs in Spain. J. Vet. Med., 53: 197-200. https://doi.org/10.1111/j.1439-0450.2006.00937.x

• Aktas M (2014). A survey of ixodid tick species and molecular identification of tick-borne pathogens. Vet. Parasitol., 200: 276-283. https://doi.org/10.1016/j.vetpar.2013.12.008

• Allison RW, Little SE (2013). Diagnosis of rickettsial diseases in dogs and cats. Vet. Clin. Pathol., 42(2): 127-144. https://doi.org/10.1111/vcp.12040

• Almazán C, González-Álvarez VH, de Mera IGF, Cabezas-Cruz A, Rodríguez-Martínez R, de la Fuentec J (2016). Molecular identification and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs in Mexico. Ticks Tick-borne Dis., 7: 276-283. https://doi.org/10.1016/j.ttbdis.2015.11.002

• Botros BA, Elmolla MS, Salib AW, Calamaio CA, Dasch GA, Arthur RR (1995). Canine ehrlichiosis in Egypt: sero-epidemiological survey. Onderstepoort J. Vet. Res., 62(1): 41-43.

• Dahmani M, Davoust B, Sambou M, Bassene H, Scandola P, Ameur T, Raoult D, Fenollar F, Mediannikov O

(2019). Molecular investigation and phylogeny of species of the Anaplasmataceae infecting animals and ticks in Senegal. Parasit. Vectors, 12: 495. https://doi.org/10.1186/s13071-019-3742-y

• Dantas-Torres F (2008). The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. Vet. Parasitol., 52: 173-185. https://doi.org/10.1016/j.vetpar.2007.12.030

• Dantas-Torres F, Chomel BB, Otranto D (2012). Ticks and tick-borne diseases: A One Health perspective. Trends Parasitol., 28: 437-446. https://doi.org/10.1016/j.pt.2012.07.003

• Dantas-Torres F, Latrofa MS, Annoscia G, Giannelli A, Parisi A, Otranto D (2013a). Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. Parasit. Vectors, 6: 213. https://doi.org/10.1186/1756-3305-6-213

• Dantas-Torres F, Capelli G, Giannelli A, Ramos RA, Lia RP, Cantacessi C, de Caprariis D, De Tommasi AS, Latrofa MS, Lacasella V, Tarallo VD, Di Paola G, Qurollo B, Breitschwerdt E, Stanneck D, Otranto D (2013b). Efficacy of an imidacloprid/flumethrin collar against fleas, ticks and tick-borne pathogens in dogs. Parasit. Vectors, 6: 245. https://doi.org/10.1186/1756-3305-6-245

• Dhliwayo S, Chihambakwe B, Taonezvi K, Chikerema SM, Tivapasi MT, Pfukenyi DM (2019). Seroprevalence of canine ehrlichiosis and microscopic screening for canine babesiosis in dogs in Harare, Zimbabwe, 2016-2017. Vet. Med. Int., Volume 2019, Article ID 4130210, 7 pages. https://doi.org/10.1155/2019/4130210

• Díaz-Sánchez AA, Corona-González B, Meli ML, Roblejo-Arias L, Fonseca-Rodríguez O, Castillo AP, Cañizares EV, Rivero EL, Hofmann-Lehmann R (2020). Molecular diagnosis, prevalence and importance of zoonotic vector-borne pathogens in Cuban shelter dogs. A preliminary study. Pathogens, 9: 901. https://doi.org/10.3390/pathogens9110901

• Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol., 51: 2145-2165. https://doi.org/10.1099/00207713-51-6-2145

• Ebani VV (2019). Serological Survey of *Ehrlichia canis* and *Anaplasma phagocytophilum* in Dogs from Central Italy: An Update (2013–2017). Pathogens, 8: 3. https://doi.org/10.3390/pathogens8010003

• Ebani VV, Rocchigiani G, Nardoni S, Bertelloni F, Vasta V, Papini RA, Verin R, Poli A, Mancianti F (2017). Molecular detection of tick-borne pathogens in wild red foxes (*Vulpes vulpes*) from Central Italy. Acta Trop. 172: 197-200. https://doi.org/10.1016/j.actatropica.2017.05.014

• El-Dakhly Kh M, Arafa W, Ghanem SS, Abdel-Fatah OR, Wahba AA (2018). Microscopic and molecular detection of *Theileria annulata* infection of cattle in Egypt. J. Adv. Parasitol., 5(2): 29-34. https://doi.org/10.17582/journal.jap/2018/5.2.29.34

• Galay RL, Manalo AAL, Dolores SLD, Aguilar IPM, Sandalo KAC, Cruz KB, Divina BP, Andoh M, Masatani T, Tanaka T (2018). Molecular detection of tick-borne pathogens in

canine population and *Rhipicephalus sanguineus* (sensu lato) ticks from southern Metro Manila and Laguna, Philippines. Parasit. Vectors, 11: 643. https://doi.org/10.1186/s13071-018-3192-y

• Ghafar MW, Amer SA (2012). 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., 3(2): 198-194. https://doi.org/10.1016/j.jare.2011.08.002

• Hamel D, Silaghi C, Lescai D, Pfist K (2012). Epidemiological aspects on vector-borne infections in stray and pet dogs from Romania and Hungary with focus on *Babesia* spp. Parasitol. Res., 110: 1537-1545. https://doi.org/10.1007/s00436-011-2659-y

• Harrus S, Waner T (2011). Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): An overview. Vet. J., 187: 292-296. https://doi.org/10.1016/j.tvjl.2010.02.001

• Horak IG, Apanaskevich DA, Kariuki EK (2013). A new species of *Rhipicephalus* (Acari: Ixodidae), a parasite of giraffes in Kenya. J. Med. Entomol., 50: 685-690. https://doi.org/10.1603/ME12257

• Huang H, Unver A, Perez MJ, Orellana NG, Rikihisa Y (2005). Prevalence and molecular analysis of *Anaplasma platys* in dogs in Lara, Venezuela. Braz. J. Microbiol., 36: 211-216. https://doi.org/10.1590/S1517-83822005000300002

• Kelly PJ, Xu C, Lucas H, Loftis A, Abete J, Zeoli F, Stevens A, Jaegersen K, Ackerson K, Gessner A, Kaltenboeck B, Wang C (2013). Ehrlichiosis, babesiosis, anaplasmosis and hepatozoonosis in dogs from St. Kitts, West Indies. PLoS One, 8 (1): e53450. https://doi.org/10.1371/journal.pone.0053450

• Latrofa MS, Dantas-Torres F, Giannelli A, Otrantoa D (2014). Molecular detection of tick-borne pathogens in *Rhipicephalus sanguineus* group ticks. Ticks Tick-borne Dis., 5: 943-946. https://doi.org/10.1016/j.ttbdis.2014.07.014

• Maggi RG, Mascarelli PE, Havenga LN, Naidoo V, Breitschwerdt EB (2013). Co-infection with *Anaplasma platys*, *Bartonella henselae* and *Candidatus Mycoplasma haematoparvum* in a veterinarian. Parasit. Vectors, 6: 103. https://doi.org/10.1186/1756-3305-6-103

• Malik MI, Qamar M, Ain Q, Hussain MF, Dahmani M, Ayaz M, Mahmood AK, Davoust B, Shaikh RS, Iqbal F (2018). Molecular detection of *Ehrlichia canis* in dogs from three districts in Punjab (Pakistan). Vet. Med. Sci., 4: 126-132. https://doi.org/10.1002/vms3.94

• Martin AR, Brown GK, Dunstan RH, Roberts TK (2005). *Anaplasma platys* an improved PCR for the detection in dogs. Exp. Parasitol., 109: 176-180. https://doi.org/10.1016/j.exppara.2004.11.007

• Nasr A, El Hariri M, Ghafar MW (2020). Detection of *Anaplasma platys* and *Ehrlichia* canis in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt; a public health concern. Vet. Med. J. Giza, 66: 1-9. https://doi.org/10.21608/vmjg.2020.157540

• Pennisi M-G, Caprì A, Solano-Gallego L, Lombardoa G, Torina A, Masucci M (2012). Prevalence of antibodies against *Rickettsia conorii*, *Babesia canis*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* antigens in dogs from the Stretto di Messina area (Italy). Ticks Tick-borne Dis., 3: 314-317. https://doi.org/10.1016/j.ttbdis.2012.10.026

• Perez M, Bodor M, Zhang C, Xiong Q, Rikihisa Y (2006). Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. Ann. N. Y. Acad. Sci., 1078: 110-

117. https://doi.org/10.1196/annals.1374.016

• Ramos RAN, Latrofa MS, Giannelli A, Lacasella V, Campbell BE, Dantas-Torres F, Otrantoa D (2014). Detection of *Anaplasma platys* in dogs and *Rhipicephalus sanguineus* group ticks by a quantitative real-time PCR. Vet. Parasitol., 205: 285-288. https://doi.org/10.1016/j.vetpar.2014.06.023

• Ribeiro CM, Matos AC, Azzolini T, Bones ER, Wasnieski EA, Richini-Pereira VB, Lucheis SB, Vidotto O (2017). Molecular epidemiology of *Anaplasma platys*, *Ehrlichia canis* and *Babesia vogeli* in stray dogs in Paraná, Brazil. Pesq. Vet. Bras., 37(2): 129-136. https://doi.org/10.1590/s0100-736x2017000200006

• Sainz Á, Roura X, Miró G, Estrada-Peña A, Kohn B, Harrus S, Solano-Gallego (2015). Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasit. Vectors, 8: 75. https://doi.org/10.1186/s13071-015-0649-0

• Salem NY, Rakha GH, Baraka TA (2014). Naturally occurring ehrlichiosis in Egyptian dogs. Iran. J. Vet. Res., 15(1): 54-57.

• Sasaki M, Omobowale O, Tozuka M, Ohta K, Matsuu A, Nottidge HO, Hirata H, Ikadai H, Oyamada T (2007). Molecular Survey of *Babesia canis* in dogs in Nigeria. J. Vet. Med. Sci., 69(11): 1191-1193. https://doi.org/10.1292/jvms.69.1191

• Selim A, Abdelhady A, Alahadeb J (2021). Prevalence and first molecular characterization of Ehrlichia canis in Egyptian dogs. Pak. Vet. J., 41(1): 117-121. http://dx.doi.org/10.29261/pakvetj/2020.061

• Spolidorio MG, Labruna MB, Zago AM, Donatele DM, Caliari KM, Yoshinari NH (2009). *Hepatozoon canis* infecting dogs in the State of Espirito Santo, southeastern Brazil. Vet. Parasitol., 163: 357-361. https://doi.org/10.1016/j.vetpar.2009.05.002

• Tanikawa A, Labruna MB, Costa A, Aguiar DM, Justiniano SV, Mendes RS, Melo ALT, Alves CJ, Azevedo SS (2013). *Ehrlichia canis* in dogs in a semiarid region of Northeastern Brazil: Serology, molecular detection and associated factors. Res. Vet. Sci., 94: 474-477. https://doi.org/10.1016/j.rvsc.2012.10.007

• Yabsley MJ, Mckibben J, Macpherson CN, Cattan PF, Cherry NA, Hegarty BC, Breitschwerdt EB, Connor TO, Chandrashekar R, Paterson T, Perea ML, Ball G, Fiesen S, Goedde J, Henderson B, Sylvester W (2008). Prevalence of *Ehrlichia canis*, *Anaplasma platys*, *Babesia canis*, *Hepatozoon canis*, *Bartonella vinsonii berkhoffi*, and *Rickettsia* spp. in dogs from Grenada. Vet. Parasitol., 151: 279-285. https://doi.org/10.1016/j.vetpar.2007.11.008

• Ybañez AP, Perezc ZO, Gaboteroc SR, Yandug RT, Kotaroa M, Inokuma H (2012). First molecular detection of *Ehrlichia canis* and *Anaplasma platys* in ticks from dogs in Cebu, Philippines. Ticks Tick-borne Dis., 3: 287-292. https://doi.org/10.1016/j.ttbdis.2012.10.032

• Ybañez RHD, Ybañez AP, Arnado LLA, Belarmino LMP, Malingin KGF, Cabilete PBC, Amores ZRO, Talle MG, Liu M, Xuan X (2018). Detection of *Ehrlichia*, *Anaplasma*, and *Babesia* spp. in dogs in Cebu, Philippines, Vet. World, 11(1): 14-19. https://doi.org/10.14202/vetworld.2018.14-19

• Zaid T, Ereqat S, Nasereddin A, Al-Jawabreh A, Abdelkader A, Abdeen Z (2019). Molecular characterization of *Anaplasma* and *Ehrlichia* in ixodid ticks and reservoir hosts from Palestine: a pilot survey. Vet. Med. Sci., 5: 230-242. https://doi.org/10.1002/vms3.150

• Zheng W, Liu M, Moumoun FA, Liu X, Efstratiou A, Liu Z, Liu Y, Tao H, Guo H, Wang G, Gao Y, Li Z, Ringo AE, C Jirapattharasate, Chen H, Xuan X (2017). First molecular detection of tick-borne pathogens in dogs from Jiangxi, China. J. Vet. Med. Sci., 79(2): 248-254. https://doi.org/10.1292/jvms.16-0484