

RESEARCH ARTICLE

Clinical, Morphological and Molecular Characterization of Canine Babesiosis and its Compatible Tick Vector in Naturally Infected Dogs, in Egypt

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Abstract

The low scientific attention in dealing with dog diseases is still present comparing to other animal species in Egypt in spite of its wide spreading as pets, hunting dogs or for other purposes. Canine babesiosis is a haemoprotozoal parasitic disease inducing significant clinical manifestations on the infected dogs. The present study aimed to accurately identify the causative species of canine babesiosis in naturally infected dogs in Egypt and its compatible tick vector. For this purpose 75 dogs including 19 dogs at Sharkia and 56 dogs at Dakahlia Governorates, Egypt were clinically examined and 20 (26.67%) dogs showed signs of fever, weakness, anorexia, pale mucous membrane and different degrees of anemia. During examination, there were 8 dogs had variant degrees of tick infestation. Further parasitological identification of 50 collected ticks showed that all the collected ticks were belong to *Rhipicephalus sanguineus* (*R. sanguineus*) species. Whole blood samples collected from all examined dogs were screened by thin blood film for presence of *Babesia* species piroplasm. Nine samples (12.0%) showed pyriform shaped protozoa inside red blood cells (RBCs). All blood samples and 5 pooled tick samples (10 ticks/each) were screened against 18s rRNA of *Babesia* species genome using semi-nested polymerase chain reaction (PCR). *Babesia canis vogeli* (*B. canis vogeli*) was the only detected species in fourteen blood samples (18.67%) and all collected tick samples. It is the first study in Egypt to use semi-nested PCR for detection of 18s rRNA of canine babesiosis in ticks collected from naturally diseased dogs.

Keywords: Canine babesiosis, *Babesia canis vogeli*, *Rhipicephalus sanguineus*, Semi-nested PCR.

Introduction

Babesiosis is a tick borne haemoprotozoal disease causing health risk infection to all animal species as well as human [1, 2]. *Babesia* species was firstly recognized by Victor Babes in the 19th century during analyzing blood specimens from sheep and cattle, while the first identification of canine babesiosis was in Italy, 1895 [3-5]. Canine *Babesia* is recognized as two species; *Babesia canis* (large 3.0–5.0 µm) and *Babesia gibsoni* (small 1.5–2.5 µm)[6]. Large *Babesia* is present in three

main subspecies, namely *Babesia canis canis*, *B. canis vogeli*, and *B. canis rossi*, the three species are antigenically distinct. *B. canis rossi* is considered the most virulent subspecies and it is predominant in southern Africa [7]. *B. canis canis*, is the predominant subspecies in Europe and Asia, while, *B. canis vogeli* was reported in the Mediterranean region, USA, Australia, Japan, Brazil and South Africa [8]. *B. gibsoni* is mainly distributed in Middle East,

Japan, Europe and USA and it causes acute disease [9].

Occurrence and distribution of each species of *Babesia* are closely related to distribution of its vector. *B. canis canis* is transmitted by *Dermacentor reticulatus* [10, 11], *B. canis vogeli* is transmitted by *Rhipicephalus sanguineus* and *B. gibsoni* is mainly transmitted by *Haemaphysalis longicornis* and *Rhipicephalus sanguineus* ticks [12]. Tick is the definitive host for *Babesia* species where the sexual replication of the protozoa occurs in its intestinal lumen then sporozoites move to salivary gland of the tick till infection of animals occur during tick bites [13]. On the other hand, many tick species act as vectors of viruses, protozoa and rickettsia causing human diseases [14-16], therefore attention should be paid for studying the identification, distribution and behavior of various tick species.

Asexual replication of *Babesia* species occurs inside RBCs of the animals by binary fission. It causes rupture of RBCs leading to haemoglobinemia, hemoglobinuria, anemia, and jaundice. Splenomegaly is the clearest pathognomonic lesion in the infected carcass. Ubah *et al.* [17] recorded pulmonary edema, testicular necrosis, hepatomegaly and kidney damage in male dogs with complicated infection of *B. canis* in addition to patched haemorrhagic inflammation of the intestines, mesentery and cardiac musculature.

The severity of the disease, prognosis and antibabesial drug response may be different for each species of *Babesia* [12]. Thus, it is important to distinctly determine the species of *canine Babesia* [18].

Thin blood film can only differentiate between large and small *Babesia* species [19], but it is difficult to detect low parasitemia [20] and distinguish between the different *Babesia* subspecies. We performed the present clinical study to determine the different species of canine babesiosis in naturally infected dogs by clinical signs, thin blood smear examination and semi-nested PCR with confirmation of the role of tick vector in the transmission of canine babesiosis.

Materials and Methods

Animals

The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt. During the period from June 2018 to September 2019, 75 dogs were clinically examined in private pet clinics at Dakahlia Governorate and in the Teaching Veterinary Hospital at the Faculty of Veterinary Medicine, Zagazig University, Sharkia Governorate, Egypt. Examined dogs were of different age, sex and breed. Previous history of signs of blood parasites and tick infestation as well as the usage of acaricides rate were recorded.

General physical examination, body temperature, pulse, respiratory rate were measured and mucous membranes were observed for any change in normal color. Dogs were carefully examined for external parasites infestation on the skin [21].

Samples

Blood samples

Blood samples were collected from ear vein for the preparation of blood films to detect piroplasm inside RBCs of infected dog according to Jain [22]. Whole blood samples were collected from cephalic vein of dogs with adding EDTA as anticoagulant for semi-nested PCR screening.

Tick samples

Fifty ticks were collected manually by forceps from their attachment sites on dog's bodies. To ensure that the mouth parts were not left behind, embedded living ticks were effectively removed by dabbing the ticks and the surrounding skin with alcohol, this relaxes the ticks and allow pulling them out intact [23]. Ticks were put in tubes containing (5 part of 70% ethyl alcohol: 3 part of glycerin). Ticks were primarily identified by naked eyes and simple microscope then were divided into two parts; one part for further parasitological identification and the other for semi-nested PCR screening.

Preparation of permanent mounts of tick for identification

Preserved tick stages were punctured with a fine needle (to evacuate contents of internal organs), heated in 10% sodium hydroxide in water bath for 15 minutes and then washed several times in cold water. After washing with water, dehydration was done by ascending concentrations of ethyl alcohol beginning from 70%, 90%, 95% and absolute ethanol for 20 minutes each. Finally, dehydrated specimens were passed through clove oil, xylol and mounted in canada balsam [24]. The collected ticks were identified according to the keys of Taylor *et al.* [25] and Bowman [26].

Molecular identification of Babesia species

DNA extraction

Extraction of DNA from blood samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH, Catalog number 51304) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample were incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol were added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit and stored at -20°C. The DNA from tick samples was extracted by the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 25

mg of the sample were incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56°C overnight. After incubation, 200 µl of AL buffer were added to the lysate, incubated for 10 min. at 72°C, then 200 µl of 100% ethanol were added to the lysate. The lysate was then transferred to silica column, centrifuged. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit and stored at -20°C.

PCR amplification

The used primers were supplied from Metabion (Germany) are listed in Table (1). The PCR was carried out in 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler. Semi-nested PCR was used to distinguish between the different species of canine *Babesia* using specific forward primers against *B. canis canis*, *B. canis vogeli*, *B. canis rossi* and *B. gibsoni*. Primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (Forward nested primer and the reverse primer) of 20 pmol concentration, 9.5 µl of water, and 1 µl of DNA template. Each run included positive *Babesia* spp. control as well as negative control (without adding DNA template) [27].

Table 1: Primers sequences, target genes (18s rRNA) and amplicon sizes

Target parasite	Primers sequences	Amplified segment (bp)	Reference
<i>Babesia</i>	F: GTCTTGTAATTGGAATGATGGTGA C R: ATGCCCCCAACCGTTCCTATTA	340	[58]
<i>Babesia gibsoni</i>	F: ACTCGGCTACTTGCCTTGTC R: ATGCCCCCAACCGTTCCTATTA	185	[58]
<i>Babesia canis vogeli</i>	F: GTTCGAGTTTGCCATTCGTT R: ATGCCCCCAACCGTTCCTATTA	192	[58]
<i>Babesia canis canis</i>	F: TGC GTTGACGGTTTGACC R: ATGCCCCCAACCGTTCCTATTA	198	[58]
<i>Babesia canis rossi</i>	F: GCTTGGCGGTTTGTTGC R: ATGCCCCCAACCGTTCCTATTA	197	[58]

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpilot100 bpladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

N.B. All diseased dogs were treated against canine babesiosis with imidocarb dipropionate 6.6mg/kg intramuscularly (IM) according to Plumb [28], fluid therapy, in addition to using acaricides to control ticks upon dog's body and surrounding environment. Owners were advised to check their pets regularly for tick

infestation with regular correct application of acaricides for dogs and their shelters.

Results

Clinical signs in the dogs

Clinical signs compatible to canine babesiosis infection such as fever (39-41°C), anorexia, dullness, weakness, red urine and pale mucous membranes were observed in 26.67% (20/75) of the examined dogs.

Parasitological examination

Blood films examination

Microscopic examination of thin stained blood smears which were made from the peripheral ear vein of examined animals revealed piroplasm of *Babesia* sp. inside RBCs in 12% (9/75) of the examined blood samples (Table 2). The piroplasms were large pyriform in shape and lies at angle to one another in side RBCs (Figure 1).

Table 2: Canine babesiosis investigation in the examined dogs

		Examined (n=75)	Infected			
			Blood film (n=9)		PCR (n=14)	
			No	%	No	%
Locality	Sharkia	19	3	15.79	3	15.79
	Dakahlia	56	6	10.71	11	19.64
Breed	German	32	7	21.88	10	31.25
	Pit pull	25	2	8	4	16
	Husky	11	0	0	0	0
	Griffon	7	0	0	0	0
Age	0-6m	13	0	0	0	0
	6m-1y	23	4	17.39	7	30.43
	1y-2y	39	5	12.82	7	17.95
Sex	Male	22	3	13.64	6	27.27
	Female	53	6	11.32	8	15.09
Tick infestation	Infested	8	8	100	8	100
	Non infested	67	1	1.49	6	8.96

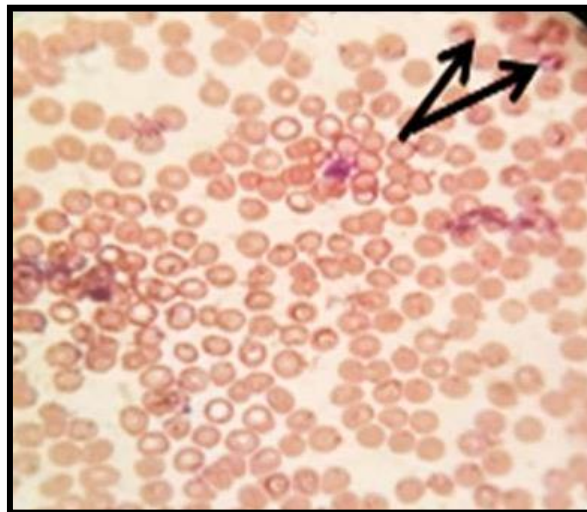


Figure 1: Stained blood film with large-sized *Babesia* species in erythrocytes of clinically diseased female german dog, 7 month of age at veterinary clinic at Dakahlia Governorate.

Morphological identification of tick

During examination; eight dogs showed varying degrees of tick infestation on their bodies. Ticks were mostly observed on the head particularly ear, axilla and on the trunk. Fifty ticks were manually retrieved directly from dog's body.

Rhipicephalus Sanguineus was the only morphologically detected group of tick. Forty-three collected ticks were adult and seven ticks were nymph. They were distinguished by reddish brown color and elongated body. The body was divided into anterior gnathosoma (or capitulum) and posterior idiosoma which carry legs. The basis of capitulum was hexagonal shape dorsally. The capitulum carried a pair of four-segmented palps, hypostomes and a pair of chelicerae. The palps were short and wider than they were long. The scutum covered the whole dorsal surface of males, while it covered a small part behind the head of nymph. This species was characterized by bifurcate coxa-1 with 2 strong spurs, comma shaped spiracles and presence of eyes and festoons (Figure 2).

Molecular detection of Babesia species

In blood samples of dogs

Whole blood samples were screened against 18s rRNA of *Babesia* sp. genome using semi-nested PCR. In the primary PCR cycle; 14 blood samples (18.67%) revealed a band of 340 bp on agarose gel which is specific for *Babesia* spp. (Table 2) (Figure 3, A). The secondary cycle of PCR used to distinguish the different species of *Canine Babesia*. A band of 192 bp was observed on agarose gel for the 14 previously positive samples which indicated only *Babesia canis vogeli* infection (Figure 3, B).

Out of fourteen positive samples, ten dogs belonged to German breed and four for Pitbull breed. Concerning age, seven dogs were aged from 6 months to 1 year and seven dogs aged 1-2 years were positive. Regarding tick infestation, eight dogs were infested with ticks, two dogs were free from tick infestation and four dogs were with previous history of tick infestation (Table 2).

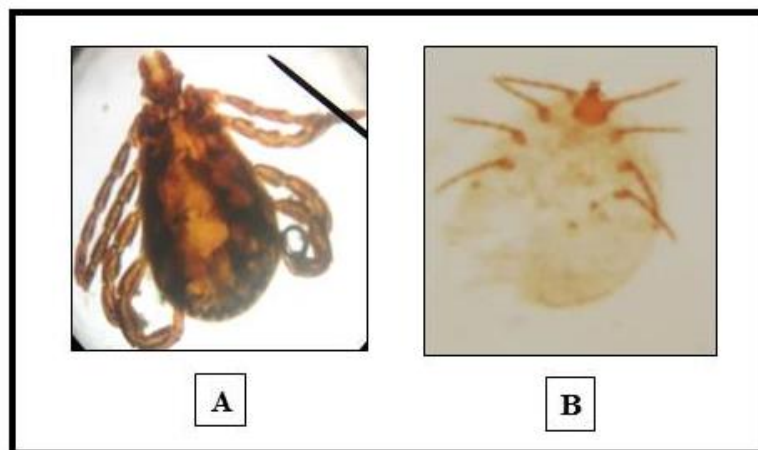


Figure 2: *Rhipicephalus sanguineus* (A) adult, (B) nymph collected from female german dog 1.5 year of age at veterinary clinic at Dakahlia Governorate.

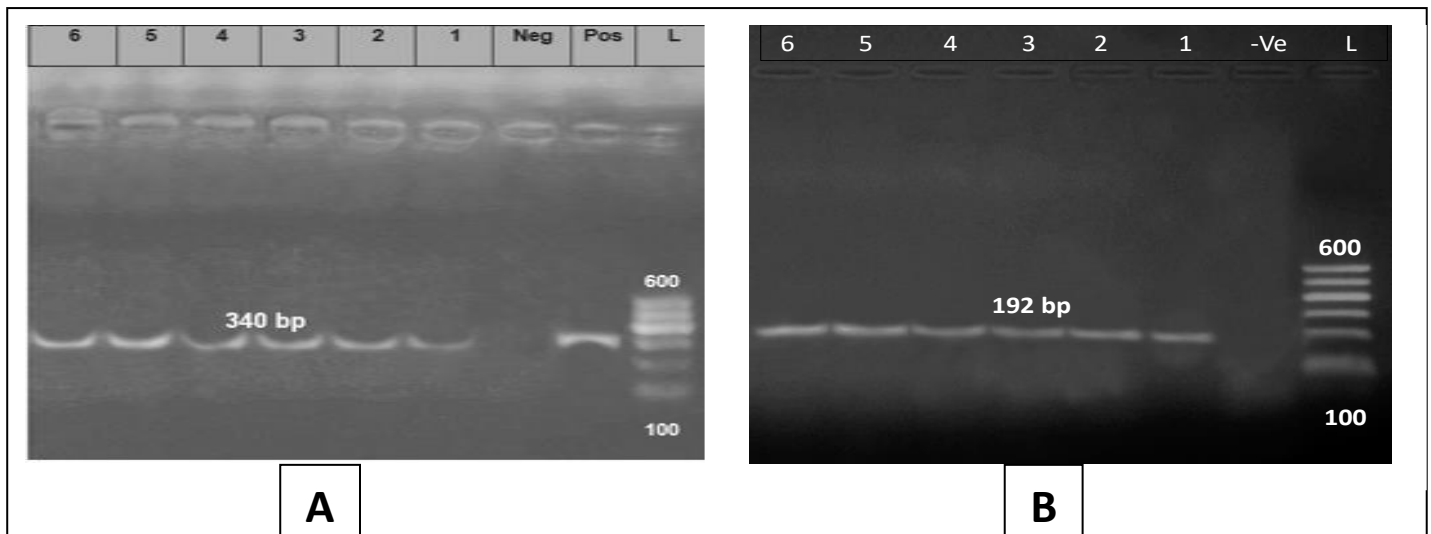


Figure 3: Agarose gel electrophoresis of the PCR products A) The primary cycle of semi-nested PCR showing a band of 340 bp for all examined samples. (pos.): from positive control of canine *Babesia*. (neg.): from negative control. (Lanes 1-5): from positive blood samples. (Lane 6): from positive tick sample. B) The secondary specific cycle of semi-nested PCR showing a specific band for *Babesia canis vogeli* of 192bp in all examined samples. (-VE): from negative control. (Lanes 1-5): from positive blood samples. (Lane 6): from positive tick sample.

Tick samples

Five pooled tick (10 ticks/each) samples were screened against 18s rRNA of *Babesia* sp. genome using semi-nested PCR. All samples revealed a band of 340 bp and 192 bp on agarose gel in the primary and secondary cycle respectively (Figure 3). The present results clarify that only *Babesia canis vogeli* was detected in the examined samples and *R. Sanguineus* is the main tick vector for transmission of canine babesiosis in Egypt.

Discussion

Babesiosis is a host specific infectious disease that threatens the life of human and all domestic animals. Canine babesiosis is caused by different species of genus *Babesia* which have different styles of geographical distribution, mode of transmission, clinical manifestations, treatment, and prognosis [13]. Therefore, it is necessary to determine different species of canine babesiosis with its vector of ticks in the infected cases in different localities. Clinical examination of 75 dogs, revealed signs that lead to suspect infection with canine

babesiosis in 20 examined dogs (26.67%). The piroplasm of *Babesia* species was detected in 12% (9/75) of the total examined dogs in stained blood smears, while semi-nested PCR detected the infection with canine babesiosis in 18.67% (14/75) of the examined dogs. The difference in the percentage of positive samples recorded by blood smear and semi-nested PCR may be attributed to the fact that *B. canis vogeli* induce chronic moderate infection with low parasitemia so it may be missed in blood smear examination [29]. Therefore, semi-nested PCR is more accurate, sensitive and rapid method for detection and identification of canine babesiosis [30].

A nearly similar percentage was reported by Singh *et al.* [31] in India who reported a percentage of 7.47% using stained blood film and 15.42% using PCR. Costa *et al.* [32] in Brazil reported a percentage of (16.1%) using the indirect immunofluorescence assay (IFA). Abdel-Rhman *et al.* [33] in Egypt recorded a lower percentage (8.5%) by blood film examination of dogs in five different governorates (Alexandria, Cairo, Giza,

Qaluobia, Kafrelsheikh). Another lower percentage (5.9%) was reported by Duh *et al.* [34] in Slovenia. In contrast, a higher percentage (57%) was reported in India by Augustine *et al.* [35] who used seminested PCR and Akande *et al.* [36] reported a percentage of 22% in Nigeria. The variances in the prevalence rates among several studies could be attributed to the species of *Babesia*, diagnostic techniques, the dog population and the country under study.

Canine babesiosis may be represented clinically as uncomplicated cases as a result of the hemolytic action of the protozoan or complicated cases due to systemic inflammatory reaction and disturbance in many organs function [37-40]. Diseased dogs under the present study showed only uncomplicated form of the disease. Elevation in body temperature, lethargy, anorexia, red urine, pale mucous membranes and different degrees of anemia were the most observed signs. Similar manifestations were reported by Salem and Farag [41], Ybanez *et al.* [42] and Islam *et al.* [43]. Severe complicated babesiosis was observed by Ubah *et al.* [17] who reported testicular degeneration for the first time in a male dog infested with *R. sanguineus*, in addition to interstitial pneumonia and cellular infiltration in the heart.

In the present study, *Rhipicephalus sanguineus* morphologically was similar with that described by Adam *et al.* [44], Jain and Jain [45], Taylor *et al.* [25] and Williams [46]. *R. sanguineus* (kennel or brown dog tick) is the main vector of *B. canis vogeli* [47]. This tick has great adaptability to many host species and many climates so it possess a wide geographical distribution [48]. Dog is the main host of *R. sanguineus* adult and nymph but it can also infest other animals [15, 49, 50] and human [51]. *R. sanguineus* ticks establish themselves in tropical, subtropical and temperate zones, so they are mainly distributed in Mediterranean areas. Hansford *et al.* [52] reported importation of *R. sanguineus* into the UK (colder area) via dogs. Other studies proved that there are two groups of *R. sanguineus*, one adapted to temperate and

another to tropical areas [53, 54]. Consequently, further molecular, sequencing and phylogenetic analysis are needed for detection and accurate recognition of the distribution of this type of ticks. Therefore, this allows successful control and treatment strategy of canine babesiosis.

Six diseased dogs had no tick infestation during examination and proved to be infected with canine *Babesiosis* by semi-nested PCR. This may be explained as after conventional treatment many dogs become chronic carriers and may show recurrent episodes of acute babesiosis [55]. Also, the brown dog ticks have cryptic behavior, this means, they are visible during seeking for a host and during their feeding but during digestion of the blood meal, molting and eggs laying; it is difficult to observe them [56].

All the infected dogs in the present study were aged from 6 months to 2 years. No infection was detected in puppies less than 6m. Singh *et al.* [31] in India found the highest prevalence of canine babesiosis infection in the age between 6 month to 1 year (30 %) comparing to (13.57%) in dogs more than 1 year and (5.88%) in puppies less than 6 months. While Abdel-Rhman *et al.* [33] in Egypt reported no significance of the age in the prevalence of canine babesiosis. Moreover they reported a higher severity of the clinical signs of the disease in puppies than adult. This difference may be due to the breeding condition as well as the number and variety of the samples. Also it was proved that the sex and breed of animals don't influence the rate of canine babesia infection although German dog appears to be more susceptible to canine babesiosis infection. The same result was reported by Atta [57] but the restriction in the samples number and breed variety can't accurate this result.

Sequencing of 18s rRNA allows the identification of different *Babesia* species [8, 34, 58]. Depending on the 18s rRNA amplification by semi-nested PCR, the genus of canine babesiosis was identified by a band of 340 pb in the primary cycle for 18.67% of

the examined samples and different species of *Babesia* could be distinguished in the secondary run and only *B. canis vogeli* was detected by a band of 192 bp. The same results were reported by Atta [57].

Conclusion

Our study confirmed that canine babesiosis in Egypt is only caused by *B. canis vogeli* and its competent vector is *R. Sanguineus*. The semi-nested PCR is more sensitive for detection *B.canis* in both dog blood and ticks. More knowledge of tick incidence, distribution, behavior and control is needed.

Conflict of interest

None of the authors have any conflict of interest to declare.

Acknowledgment

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References

- [1] El-Bahnasawy, M.M.; Khalil, H. and Morsy, T.A. (2011): Babesiosis in an Egyptian boy aquired from pet dog, and a general review. J Egypt Soc Parasitol, 41(1): 99-108.
- [2] Schnittger, L.; Rodriguez, A.E.; Florin-Christensen, M. and Morrison, D.A. (2012): Babesia: a world emerging. Infect Genet Evol, 12(8): 1788-809.
- [3] Anand, K.; Anand, A. and Kashyap, A. (2015): Dr. Victor Babes, Discoverer of Babesia. J Assoc Physicians India, 63(3): 80-1.
- [4] Uilenberg, G. (2006): Babesia—a historical overview. Vet Parasitol, 138(1-2): 3-10.
- [5] Amici, R.R. (2001): The history of Italian parasitology. Vet Parasitol, 98(1-3): 3-30.
- [6] Jefferies, R.; Ryan, U.M.; Muhnckel, C.J. and Irwin, P.J. (2003): Two species of canine Babesia in Australia: detection and characterization by PCR. J Parasitol, 89(2): 409-12.
- [7] Matjila, P.T.; Leisewitz, A.L.; Jongejan, F. and Penzhorn, B.L. (2008): Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. Vet Parasitol, 155(1-2): 152-7.
- [8] Matjila, P.; Penzhorn, B.; Bekker, C.; Nijhof, A. and Jongejan, F. (2004): Confirmation of occurrence of Babesia canis vogeli in domestic dogs in South Africa. Vet Parasitol, 122(2): 119-25.
- [9] Conrad, P.; Thomford, J.; Yamane, I.; Whiting, J.; Bosma, L.; Uno, T.; Holshuh, H. and Shelly, S. (1991): Hemolytic anemia caused by Babesia gibsoni infection in dogs. J Am Vet Med Assoc, 199(5): 601-5.
- [10] Mehlhorn, H.; Schein, E. and Voigt, W. (1980): Light and electron microscopic study on developmental stages of Babesia canis within the gut of the tick Dermacentor reticulatus. J Parasitol, 66: 220-8.
- [11] Varloud, M.; Liebenberg, J. and Fourie, J. (2018): Early Babesia canis transmission in dogs within 24 h and 8 h of infestation with infected pre-activated male Dermacentor reticulatus ticks. Parasites Vectors, 11(1): 41.
- [12] Solano-Gallego, L. and Baneth, G. (2011): Babesiosis in dogs and cats—expanding parasitological and clinical spectra. Vet Parasitol, 181(1): 48-60.
- [13] Solano-Gallego, L.; Sainz, Á. Roura, X.; Estrada-Peña, A. and Miró, G. (2016): A review of canine babesiosis: the European perspective. Parasites Vectors, 9(1): 336.
- [14] Gilot, B.; Laforge, M.; Pichot, J. and Raoult, D. (1990): Relationships between the Rhipicephalus sanguineus complex ecology and Mediterranean spotted fever

- epidemiology in France. *Eur J Epidemiol*, 6(4): 357-62.
- [15] Labruna, M. (2004): *Biologia-ecologia de Rhipicephalus sanguineus (Acari: ixodidae)*. *Rev Bras Parasitol Vet*, 13(S1): 123-4.
- [16] Parola, P. and Raoult, D. (2001): Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin Infect Dis*, 32(6): 897-928.
- [17] Ubah, A.S.; Abalaka, S.E.; Idoko, I.S.; Obeta, S.S.; Ejiofor, C.E.; Mshelbwala, P.P.; Omeje, J.N. and Ajayi, I.E. (2019): Canine babesiosis in a male Boerboel: Hematobiochemical and anatomic pathological changes in the cardiorespiratory and reproductive organs. *Veterinary and Animal Science*, 7: 100049.
- [18] Collett, M. (2000): Survey of canine babesiosis in South Africa. *J S Afr Vet Assoc*, 71(3): 180-6.
- [19] Solano-Gallego, L.; Trotta, M.; Carli, E.; Carcy, B.; Caldin, M. and Furlanello, T. (2008): *Babesia canis canis* and *Babesia canis vogeli* clinicopathological findings and DNA detection by means of PCR-RFLP in blood from Italian dogs suspected of tick-borne disease. *Vet Parasitol*, 157(3-4): 211-21.
- [20] Böhm, M.; Leisewitz, A.L.; Thompson, P.N. and Schoeman, J.P. (2006): Capillary and venous *Babesia canis rossi* parasitaemias and their association with outcome of infection and circulatory compromise. *Vet Parasitol*, 141(1-2): 18-29.
- [21] Chauhan, R.S. and Agarwal, D.K. (2008): *Textbook of Veterinary Clinical and Laboratory Diagnosis*. 2nd Ed. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd.
- [22] Jain, N.C. (1986): *Schalm's veterinary hematology*. 4th ed. Philadelphia, London: Lea & Febiger.
- [23] Bowman, D. (1999): *Georgis Parasitology for Veterinarians*. 7th ed. Philadelphia: W. B. Saunders Company.
- [24] Soulsby, E. (1968): *Helminths, arthropods and protozoa of domesticated animals*, Balliere Tindall. 7th edition ed. London: Balliere, Tindall and cassell.
- [25] Taylor, M.; Coop, R. and Wall, R. (2007): *Veterinary Parasitology*. 3rd ed. Oxford: Blackwell Publishing.
- [26] Bowman, D.D. (2009): *Georgis' parasitology for veterinarians*. 9th ed. China: an imprint of Elsevier Inc., Saunders.
- [27] Casati, S.; Sager, H.; Gern, L. and Piffaretti, J.C. (2006): Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Ann Agric Environ Med: AAEM*, 13: 65-70.
- [28] Plumb, D. (2015): *Plumb's Veterinary Drug AD= atopic dermatitis Handbook*. 8th edition. Ames, IA: Wiley-Blackwell.
- [29] Irwin, P. and Hutchinson, G. (1991): Clinical and pathological findings of *Babesia* infection in dogs. *Aust Vet J*, 68(6): 204-9.
- [30] Moraes, P.H.G.; Rufino, C.P.; Reis, T.; Aguiar, D.C.F.; Meneses, A.M.C. and Gonçalves, E.C. (2014): Optimization of a molecular method for the diagnosis of canine babesiosis. *Rev Bras Parasitol V*, 23(1): 105-8.
- [31] Singh, A.; Singh, H.; Singh, N.K.; Singh, N.D. and Rath, S.S. (2014): Canine babesiosis in northwestern India: molecular detection and assessment of risk factors. *Biomed Res. Int.*, 2014: 741785-5 pages.
- [32] Costa, A.P.d.; Costa, F.B.; Labruna, M.B.; Silveira, I.; Moraes-Filho, J.; Soares, J.F.; Spolidorio, M.G. and Guerra, R.d.M.S.N. (2015): A serological and molecular survey of *Babesia vogeli*, *Ehrlichia canis* and *Rickettsia* spp. among dogs in the state of Maranhão, northeastern Brazil. *Rev Bras Parasitol V*, 24(1): 28-35.

- [33] Abdel-Rhman, A.A.; Hegazy, Y.M. and Al-Gaabary, M.H. (2015): Canine Babesiosis in an Endemic Area of the Middle East; Causative Agent Identification, Prevalence Estimation and Risk Factors Determination. Alexandria Journal for Veterinary Sciences, 47(1): 104-12.
- [34] Duh, D.; Tozon, N.; Petrovec, M.; Strašek, K. and Avšič-Županc, T. (2004): Canine babesiosis in Slovenia: molecular evidence of *Babesia canis canis* and *Babesia canis vogeli*. Vet Res, 35(3): 363-8.
- [35] Augustine, S.; Sabu, L. and Lakshmanan, B. (2017): Molecular identification of *Babesia* spp. in naturally infected dogs of Kerala, South India. J Parasit Dis, 41(2): 459-62.
- [36] Akande, F.A.; Adebowale, A.F.; Takeet, M.I. and Omisile, O.K. (2017): Prevalence of *Babesia* species in hunting dogs in Ogun State South West Nigeria. Alexandria Journal for Veterinary Sciences, 54(1): 1-7.
- [37] Crnogaj, M.; Cerón, J.J.; Šmit, I.; Kiš, I.; Gotić, J.; Brkljačić, M.; Matijatko, V.; Rubio, C.P.; Kučer, N. and Mrljak, V. (2017): Relation of antioxidant status at admission and disease severity and outcome in dogs naturally infected with *Babesia canis canis*. BMC Vet Res, 13(1): 114.
- [38] Matijatko, V.; Mrljak, V.; Kiš, I.; Kučer, N.; Foršek, J.; Živičnjak, T.; Romić, Ž.; Šimec, Z. and Ceron, J.J. (2007): Evidence of an acute phase response in dogs naturally infected with *Babesia canis*. Vet Parasitol, 144(3-4): 242-50.
- [39] Schetters, T.; Moubri, K. and Cooke, B.M. (2009): Comparison of *Babesia rossi* and *Babesia canis* isolates with emphasis on effects of vaccination with soluble parasite antigens: a review. J S Afr Vet Assoc, 80(2): 75-8.
- [40] Welzl, C.; Leisewitz, A.; Jacobson, L.; Vaughan-Scott, T. and Myburgh, E. (2001): Systemic inflammatory response syndrome and multiple-organ damage/dysfunction in complicated canine babesiosis. J S Afr Vet Assoc, 72(3): 158-62.
- [41] Salem, N. and Farag, H. (2014): Clinical, hematologic, and molecular findings in naturally occurring *Babesia canis vogeli* in Egyptian dogs. Vet Med Int, 2014(4): 270345.
- [42] Ybanez, R.H.D.; Ybanez, A.P.; Arnado, L.L.A.; Belarmino, L.M.P.; Malingin, K.G.F.; Cabilete, P.B.C.; Amores, Z.R.O.; Talle, M.G.; Liu, M. and Xuan, X. (2018): Detection of Ehrlichia, Anaplasma, and Babesia spp. in dogs of Cebu, Philippines. Vet World, 11(1): 14-9.
- [43] Islam, S.T.; Firdos, W.U.; Sheikh, A.A.; Ganaie, M.Y. and Baghat, R. (2017): Hemato biochemical alterations and therapeutic management of babesiosis in a Pit bull Dog: A case study. Pharma Innov J. 6(11): 632-4.
- [44] Adam, K.M.G.; Paul, J. and Zaman, V. (1971): Medical and veterinary protozoology. An illustrated guide. Edinburgh, london: Churchill Livingstone.
- [45] Jain, P. and Jain, A. (2006): Textbook of Veterinary Entomology and Acarology. 1st ed. India: Jaypee Brothers Medical Publishers.
- [46] Williams, R.E. (2009): Veterinary entomology: livestock and companion animals. 1st ed. Boca Raton, Florida, USA: CRC Press., Taylor and Francis group.
- [47] Uilenberg, G.; Franssen, F.; Perie, N. and Spanjer, A. (1989): Three groups of *Babesia canis* distinguished and a proposal for nomenclature. Vet Q, 11(1): 33-40.
- [48] Mentz, M.B.; Trombka, M.; SILVA, G.L.d. and Silva, C.E. (2016): *Rhipicephalus sanguineus* (ACARI: IXODIDAE) biting a human being in Porto Alegre city, Rio Grande do Sul, Brazil. Rev Inst Med Trop Sao Paulo, 58: 35.
- [49] Dantas-Torres, F. and Otranto, D. (2015): Further thoughts on the taxonomy

- and vector role of *Rhipicephalus sanguineus* group ticks. *Vet Parasitol*, 208(1-2): 9-13.
- [50] Walker, A. (2000): The Genus *Rhipicephalus* (Acari, Ixodidae): A Guide to the Brown Ticks of the World; Jane B. Walker, James E. Keirans and Ivan G. Horak. *Trop Anim Health Prod*, 32(6): 417.
- [51] Dantas-Torres, F.; Figueredo, L.A. and Brandão-Filho, S.P. (2006): *Rhipicephalus sanguineus* (Acari: Ixodidae), the brown dog tick, parasitizing humans in Brazil. *Rev Soc Bras Med Trop*, 39(1): 64-7.
- [52] Hansford, K.M.; Pietzsch, M.E.; Cull, B. and Medlock, J.M. (2014): Importation of *R. sanguineus* into the UK via dogs: tickborne diseases. *Vet Rec*, 175(15): 385-6.
- [53] Moraes-Filho, J.; Marcili, A.; Nieri-Bastos, F.A.; Richtzenhain, L.J. and Labruna, M.B. (2011): Genetic analysis of ticks belonging to the *Rhipicephalus sanguineus* group in Latin America. *Acta Trop*, 117(1): 51-5.
- [54] Szabó, M.P.; Mangold, A.J.; João, C.F.; Bechara, G.H. and Guglielmone, A.A. (2005): Biological and DNA evidence of two dissimilar populations of the *Rhipicephalus sanguineus* tick group (Acari: Ixodidae) in South America. *Vet Parasitol*, 130(1-2): 131-40.
- [55] Schoeman, J.P. (2009): Canine babesiosis. *Onderstepoort J Vet Res*, 76(1): 59-66.
- [56] Tian, Y.L., C.C. and Kaufman, P.E. (2018): a common name: brown dog tick. Scientific name: *Rhipicephalus sanguineus* Latreille (Arachnida: Acari: Ixodidae). University of Florida.
- [57] Atta, H.S.F. (2012): Epidemiological, diagnostic and therapeutical studies on canine babesiosis [M.V.SC. thesis]: Faculty of Veterinary Medicine, Cairo University.
- [58] Birkenheuer, A.J.; Levy, M.G. and Breitschwerdt, E.B. (2003): Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. *J Clin Microbiol*, 41(9): 4172-7.

الملخص العربي

دراسات إكلينيكية وتوصيفية وجزيئية على بابزيا الكلاب والقراد الناقل للمرض في الكلاب، مصر وفاء محمد اسماعيل النشوي¹، إيمان أحمد علي حسانين^{2*}، عبد الكريم منصور مرسى³، ريهام جمال أبو العلا²

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مازال التعامل مع أمراض الكلاب في مصر قليلاً نوعاً ما مقارنة بالحيوانات الأخرى على الرغم من انتشاره على نطاق واسع كحيوانات أليفة أو كلاب صيد أو لأغراض أخرى. بابزيا الكلاب هو مرض تسببه أوليات تعيش في الدم ويسبب أعراض إكلينيكية في الكلاب المصابة. تهدف الدراسة الحالية إلى التعرف على الاعراض الإكلينيكية لمرض البابزيا في الكلاب والقراد الناقل لها في مصر. لهذا الغرض، تم فحص 75 كلباً (19 كلباً من محافظة الشرقية و 56 كلباً من محافظة الدقهلية)، وأظهرت الفحوصات الإكلينيكية أن عشرون كلباً بنسبة 26.67٪ عليهم علامات منفصلة من الحمى، الضعف، فقدان الشهية، الأغشية المخاطية الشاحبة ودرجات مختلفة من فقر الدم. أثناء الفحص، كان هناك 8 كلاب لديهم درجات مختلفة من الإصابة بالقراد. أثناء الفحص تم جمع خمسون قراد من ثمانية كلاب مصابة بدرجات مختلفة، وبالفحص المجهرى وجد ان هذا النوع ينتمي الى ريبيسيفاليس سينجوينس (*Rhipicephalus sanguineus*). ولمعرفة أنواع فصائل بابزيا الكلاب تم جمع عينات الدم من كل الكلاب التي تم فحصها ثم عمل مسحات الدم الشريحية المصبوغة فأظهرت بروتوزوا كمثرى الشكل داخل كرات الدم الحمراء في تسع عينات (12%). ولمعرفة الخصائص الجينية لجينوم البابزيا من عينات الدم والقراد باستخدام تفاعل البلمرة المتسلسل شبه المتداخل (Seminested PCR) تم معرفة الوزن الجزيئي لنواتج إستخلاص الحمض النووي (DNA) اعتماداً على منطقة 18s rRNA في 14 (18.67٪) عينة دم و عينات قراد و تم الكشف عن بابزيا كانز فوجيلي (*Babesia canis vogeli*) فقط في العينات التي تم فحصها. إنها أول دراسة في مصر تستخدم تفاعل البلمرة شبه المتداخل للكشف عن 18s rRNA من بابزيا الكلاب في القراد التي تم جمعها من الكلاب المصابة.