Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

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Genetic characterization and pathogenic effects of *Hepatozoon canis* infection in police dogs in Egypt

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Abstract

Background *Hepatozoon canis* is a protozoan parasite transmitted to dogs through ingesting the arthropod vector (hard ticks), which contains mature protozoal oocysts harboring infectious sporozoites.

Aims This study aims to evaluate the blood parameters, biochemical assays and histopathological appraisal of infected police dogs with *Hepatozoon canis*, from kennels in the police academy of Egypt during 2020–2021.

Methods Red blood cells count, hemoglobin, hematocrit, blood platelets and white blood cells count from collected blood samples were analyzed, and serum albumin, creatinine, urea, aspartate aminotransferase and alanine aminotransferase were analyzed from serum samples. Polymerase chain reaction amplified the 18S ribosomal RNAgene of the *Hepatozoon* species for genetic analysis, and the deoxyribonucleic acid products were sequenced and added to GenBank.

Results The present study resulted in 5% of the police dog population being infested with *Rhipicephalus sanguineus*. This study registered the sequences of the *Hepatozoon canis* 18S ribosomal RNAgene in Egypt for the first time in Genbank (MW362244.1–MW362245.1). The biochemical assay revealed that the parasite severely affected the protein, significantly increasing serum albumin in positive polymerase chain reaction testing dogs.

Conclusion A thorough inspection discovered that 100 police dogs had clinical symptoms like fever, emaciation and anemia, while the other 200 were healthy and had no evident clinical indicators.

Keywords *Hepatozoan canis*, *Rhipicephalus sanguineus*, Police dogs, Genetic characterization, Protozoa of dogs

1 Background

Numerous parasites can harm dogs' health and well-being because they are hosts for many of them. One of the most prevalent diseases in dogs is tick infestation; these canines are infected by several protozoan parasites, including *Babesia canis*, *Hepatozoan canis (H. canis*) and *Ehrlichia canis* (*E. canis*) [23]. Hepatozoonosis, a canine vector-borne illness caused by *H. canis,* has lately been discovered in South and North America [9]. Dogs from tropical, subtropical or temperate climes are more likely to develop autochthonous infections with *H. canis*, and the three-host tick species *Rhipicephalus sanguineus (R. sanguineus*) serves as their primary vector for transmission [26, 27, 43]. In contrast to other arthropod-transmitted diseases, this one is contracted by eating infected ticks rather than by tick bites. The sporozoite will be released in the stomach of dogs that unintentionally consume the brown dog tick (*R. sanguineus*), which has a sporocyst stage that forms following sporogony

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[2]. Sporozoites that have been transmitted by a tick bite pass via mononuclear cells and travel through the blood or lymph to the visceral organs of their vertebrate hosts, including the bone marrow, spleen and lymph nodes, where they mature into the meront stage [37, 9]. Canines with *H. canis* infections can present with anything from severe and potentially fatal clinical symptoms, such as acute lethargy, cachexia and anemia, to asymptomatic, in otherwise healthy canines. Merozoites infect leukocytes, but the protozoan's later-found gamont stage (which is infectious for ticks) was discovered earlier [43]. Infection with *H. canis* in dogs is primarily asymptomatic, but it can occasionally result in a condition marked by cachexia, lethargy and anemia that can be mild to lethal [47]. In dogs with high parasitemia, the condition can be severe and cause anemia, lethargy and cachexia in addition to other clinical symptoms ranging from asymptomatic (caused by low parasitemia) to severe (anemia) and exhibiting signs of cachexia and cachexia. However, the most common clinical symptoms in infected dogs include anorexia, fever, depression, weight loss and lymphadenopathy [30, 44]. Although there is a chance for mild nonregenerative anemia, leucocytosis with neutrophilia and monocytosis, or mild thrombocytopenia, hematological abnormalities in hepatozoonosis are less severe [18]. Lethargy, fever, anorexia, weight loss, lymphadenomegaly and anemia are examples of severe clinical symptoms that are typically linked to a high parasite load.

The parasite can be seen in histopathological specimens, where micromerozoites create the typical "wheel spoke" pattern of mature meronts by aligning in a circle around a central opaque core. Polymerase chain reaction (PCR), one molecular approach, has recently evolved above other techniques due to its increased sensitivities and specificities for detecting the target pathogens in peripheral blood [10]. In addition, sequence analysis has been used for identifying and diagnosing epidemiological studies of *Hepatozoon* infections [34, 29]. Therefore, this study aimed to morpho-molecular identification using the 18S ribosomal RNA (18S rRNA) gene of *H. canis* that infects the police dogs at K9 kennels in a police academy in Egypt. In addition to genetic characterization and sequencing, they constructed phylogenetic analysis. Biochemical assays and blood parameters were examined. Histopathological alternations of the infected tissues from freshly dead dogs were studied.

2 Methods

2.1 Sample collection

2.1.1 Investigated dogs

Between January 2020 and February 2021, 300 police dogs were examined from the K9 kennels at the Police Academy in Egypt. The examined dogs were between 2.5 and 12 years old. For morphological identification of the protozoan parasite, blood samples were analyzed. *This study was approved by the ethical committee of the animal care and use of the Faculty of Veterinary Medicine, Cairo University, with number Vet Cu 01122022624.*

2.1.2 Ticks

A total of 300 ticks (adult male, female and nymph stage) were collected from one hundred dogs located in K9 kennels at the police academy of Egypt. The method of collecting ticks was done according to Abdullah et al. [1]. The preserved ticks are in 70% ethyl alcohol for identification [15].

2.1.3 Blood samples

Three hundred blood samples were taken from the sick police dogs (K9). A thorough inspection discovered that 100 police dogs had clinical symptoms like fever, emaciation and anemia, while the other 200 were healthy and had no evident clinical indicators. Each dog's cephalic vein was used to draw blood, which was then placed in a tube with ethylene diamine tetra acetic acid (EDTA). Additionally, they drew blood into simple test tubes to obtain serum for biochemical analysis.

2.2 Parasitological examination

The blood smears were air dried, methanol absolute was used to fix them, and Giemsa stain was used to color them. Under a light microscope (X40 and 100) (OLYMPUS CX41), Soulsby [49] and Zaki et al. [52] examined the dyed blood smears to evaluate whether *H. canis* infection was present.

2.3 Hematological and biochemical analysis of blood infected with *H. canis*

To identify the various blood parameters, red blood cells (RBCs) count, hemoglobin (Hb), hematocrit (Hct), blood platelets (PLT) count and white blood cells (WBCs) count [25] potentially alter during canine hepatozoonosis infection. Hematological analysis was performed on 300 whole blood samples using EDTA (idexx-vet. auto read-USA). Additionally, serum was submitted to spectrophotometric techniques employing (idexx-catalyst-USA) to determine serum albumin, creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [4–7, 5, 6]. At the Egyptian police academy's K9 lab, they completed the complete hematological and biochemical analysis of blood.

2.4 Genetic characterization of *H. canis*

PCR was utilized to amplify the 18S rRNA gene of the hepatozoon species, and the deoxyribonucleic acid

(DNA) produced was subsequently sequenced. DNA extracted from blood samples taken from police dogs ($n=100$). Utilized an amplifying primer set for the *Hepatozoon* 18S rRNA gene to determine the DNA sequences of the PCR products from all samples [13, 17, 41, 45]. The DNA was extracted from 2ml blood using the QIAGEN DNA blood kits by the aid of the automatic extraction tool QIAcube. One sample of DNase/RNase-free distilled water was used as a blind control in the DNA extraction cycle. The forward primers Hep-F (5′-ATA CAT GAG CAA AAT CTC AAC-3′) and Hep-R (5′-CTT ATT ATT CCA TGC TGC AG-3′) are used to amplify the whole 18S rRNA gene under the circumstances outlined by [19, 41]. The amplified sequence gene was obtained from a 700-bp fragment of the 18S rRNA gene. The following procedures were used for amplification: 40 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s and chain extension at 72 °C for 90 s. The initial denaturation took place for 5 min at 94 °C. The final stage was performed with a 5-min final extension at 72 °C [13, 22, 50].

The assembled sequences were bioedit using the Edit Seq tools (Lasergene; DNASTAR, INC., Madison, WI, USA) using Edit Seq tools.

2.5 Histopathology

The necropsied liver and spleen samples from fifteen police dogs that had recently died of infection were preserved in 10% neutral buffered formalin. Tissue specimens were routinely processed, embedded in paraffin and cut into 5 µm sections. Hematoxylin and eosin (H&E) staining was performed on tissue sections [12]. We used an Olympus BX43 light microscope with a DP-27 Olympus camera to analyze stained tissue slides.

2.6 Statistical analyses

All data were expressed as mean ± S.E. (standard error) [7]. Statistical comparison between the mean of the different groups (positive and negative PCR testing dogs) was made by independent samples T-Test. The sensitivity and specificity of histopathology were compared with PCR (gold standard) by the Chi-square test. Values of $P \leq 0.05$ were considered statistically significant. SPSS version 26 was utilized for the analyses (SPSS Inc, Chicago, IL, USA). Statistics were judged significant at $P \leq 0.05$ or higher [33, 51].

3 Results

3.1 Identification of ticks

Examination of the three hundred police dogs revealed that 200 were healthy and without tick infestation, and one hundred were infested with ticks and showed clinical signs. The identified tick specimens as *R. sanguineus* with an infestation rate (30%). The detected male ticks were characterized by a dark brown color and the presence of grooves on the dorsal surface of the body. In contrast, the female ticks were characterized by scutum covering the anterior body and a short hypostome (wider than long). All developmental stages of ticks were determined (adult male, female and nymph stage) from the infested police dogs (Fig. 1).

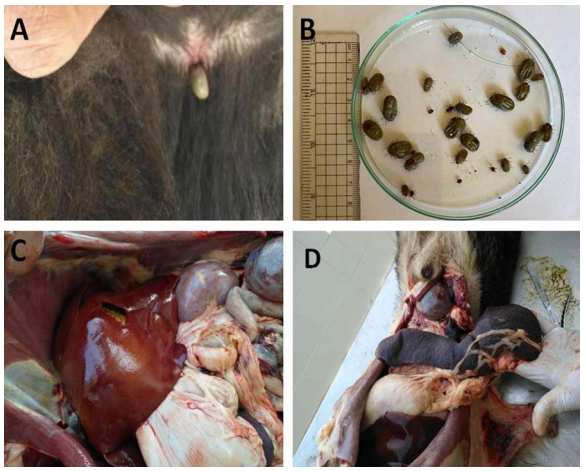

Fig. 1 **A** A police dog infested with brown dog ticks. **B** The collected male and female *R. sanguineus* were widely distributed brown dog ticks. **C** Postmortem examination of dogs infected with *H. canis* showing hemorrhagic liver. **D** Postmortem examination of dogs infected with *H. canis* showing enlarged spleen (splenomegaly)

3.2 Ticks distribution

The prevalence rate of infestation with ticks investigated was 30.0% on police dogs. The detected *R. sanguineus* ticks from soft parts of dogs from the neck, chest area and inner sides of the forelegs. German shepherd breed of dogs was more sensitive to tick infestation with a prevalence of 88% than Malino, 12%. The intensity rates of infestation differ ranged from mild (less than ten ticks on the body) (66%), moderate (10–20 ticks on the body) (23%) and severe (more than 20 ticks on the body) (11%) (Table 1). Regarding the gender-investigated dogs, a male was the more prevalent by 90% of the total sample, followed by a female (10%).

3.3 Blood film examination

Microscopically examined blood film with Giemsa-stained to determine the prevalence of infection (5.0%) with *H. canis*. No other hemoparasites were diagnosed in the blood smears.. A microscopic field of infected dogs included 2 and 5 *H. canis* gamonts per field of parasites with oil immersion lens. In the cytoplasm of the leukocyte, a capsule encased the protozoan. These gamonts are clear to pale blue and oval to elliptical structures

Table 1 Erythrogram and leukogram parameters in positive and negative *H.canis* PCR testing dogs

Parameters	Negative PCR ($n=90$)	Positive PCR ($n=10$)	t (df)	P value
RBCs (10^6 cell/µl blood)	6.73 ± 0.101	5.61 ± 0.597	3.124 (98)	0.002
WBCs (10^3 /µl)	9.06 ± 0.429	12.29 ± 1.613	− 2.325 (98)	0.022
Hb (g/dl)	13.75 ± 0.139	12.38 ± 0.840	2.764 (98)	0.007
Hct (%)	39.50 ± 0.304	38.14 ± 1.994	1.216 (98)	0.227
PLT (10^3 /µl)	202.36 ± 5.643	206.00 ± 14.621	− 0.207 (98)	0.837

Data are presented as mean ± SE, P value ≤ .05 are statistically significant (Independent Samples T-test)

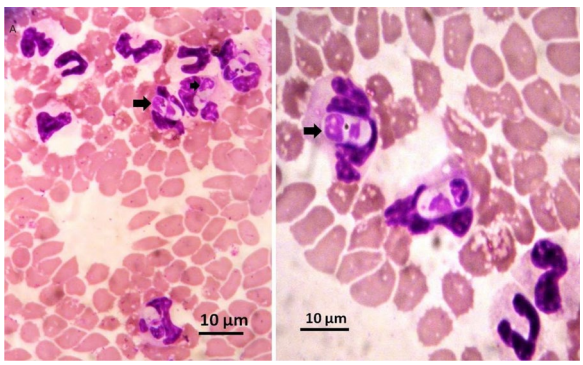

Fig. 2 The thin stained blood smears from police dogs revealed the presence of *H. canis* gamonts, ellipsoid-shaped gamont in leukocyte cells. The mature gamonts of *H. canis* showed gamonts ellipsoidal within neutrophils, where enlargement of the host cell and displacement of the host cell nucleus (× 100)

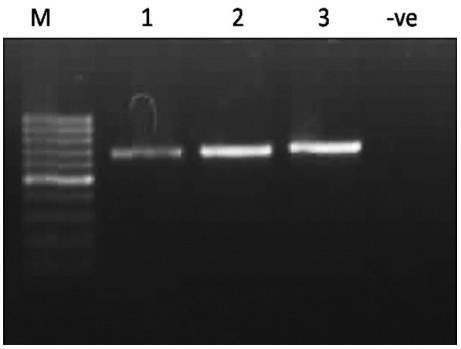

Fig. 3 Purified PCR products from *H. canis* at 700 bp

found in monocytes, neutrophils or cytoplasm. Also, a host cell enlarges, and its nucleus is displaced, as seen in blood smears; the mature gamonts are ellipsoidal (intracytoplasmatic ellipsoid-shaped) within neutrophils. The organism typically moves the nucleus to one side of the cell. *H. canis* gamonts' determined dimensions were 10.1–10.9 mm and 5.3–6.6 mm (Fig. 2).

3.4 Molecular characterization

The universal primers indicated earlier in this work were successfully used to amplify the *H. canis* 18S rRNA region analysis. This blood protozoan parasite produced purified PCR products that were immediately sequenced and assembled at 700 bp (Fig. 3).

3.5 BLAST analysis and phylogenetic tree

10% of the studied dogs had *H. canis* positive as determined by PCR and amplicon sequencing. In the current study of *H. canis* 18S fragment sequences, there was slight variation among the *H. canis* sequences with others previously registered in GenBank. The registered presently obtained *H. canis* sequences in GenBank with the specified accession codes (MW362244.1 and MW362245.1). In BLAST analysis, clustered together with other *H. canis* sequences deposited in GenBank, all sequences of investigated piroplasms were 99.98–100.0% nucleotide identical to *H. canis* from dogs. The present sequence (MW362244.1) revealed that 100.0% nucleotide identity to sequences from dogs in KJ605145.1, MF142765.1, LC 018194.1, KU535868.1, LC018203.1, KP233215.1, JX027010.1, FJ497018.1, MZ476776.1 and KF692039.1 in India, Qatar, Portugal, Pakistan, Portugal, Brazil, Nigeria, Croatia and Brazil, respectively, while revealing 99.98% nucleotide identity to sequences from dogs in India (MG050161.1 and KX377968.1) (Fig. 4). Moreover, the obtained sequence (MW362245.1) revealed that 100.0% nucleotide identity to sequences from dogs in Nigeria and India: JQ976623.1 and MG018464.1.

3.6 Hematological and biochemical analysis

The erythrogram showed that RBCs and Hb were significantly lower in PCR-positive *H. canis* testing dogs (10%) compared to the negative *H. canis* testing dogs. On the other hand, Hct and PLT showed a nonsignificant difference in positive PCR testing dogs compared with negative PCR testing dogs. Results of the leukogram showed that WBCs were significantly higher in PCR-positive *H. canis* testing dogs compared to the negative *H. canis* testing dogs, as shown in Table 1.

Serum albumin showed a significant increase in positive PCR testing dogs compared with negative PCR testing dogs. On the other hand, AST and ALT showed no significant difference between negative and positive PCR testing dogs, as shown in Table 2**.**

As shown in Table 3, the kidney function tests performed on the studied dogs revealed no statistical difference in the levels of urea and creatinine between the dogs with negative and positive PCR results.

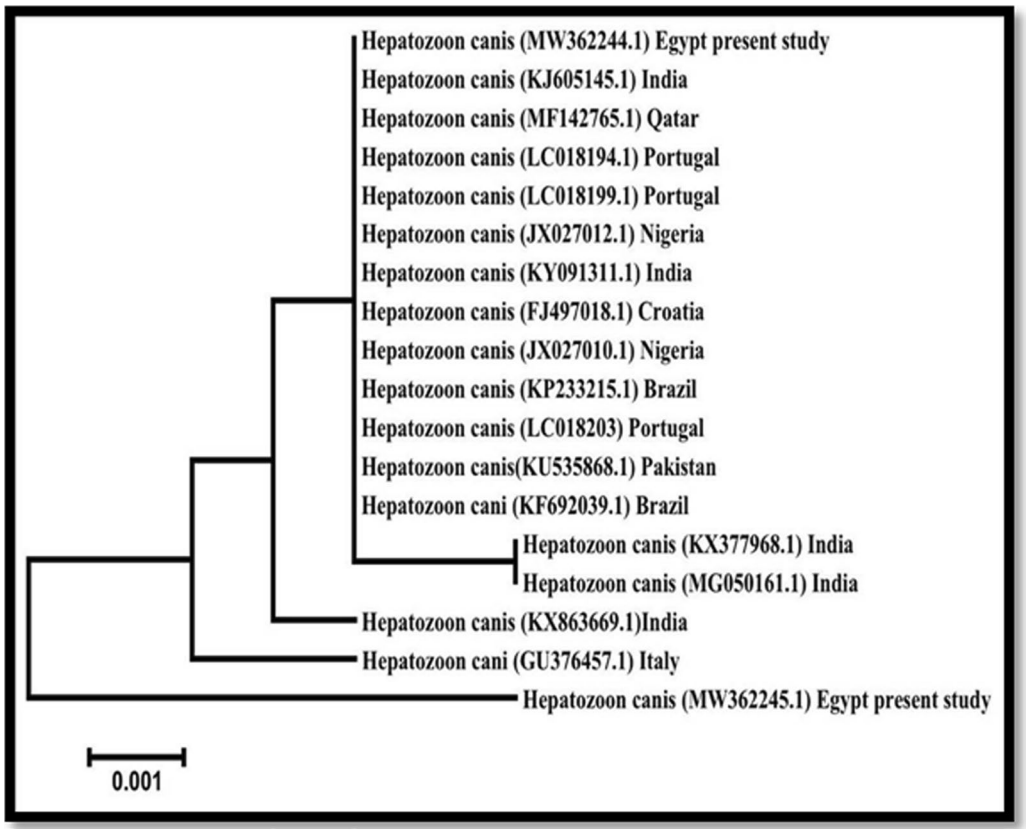

Fig. 4 Phylogenetic tree of the 18S rRNA gene sequences of *H. canis* created using the neighbor-joining method. Sequences with accession numbers have been taken from GenBank for comparison

Table 2 Results of liver function in positive and negative *H. canis* PCR testing dogs

Parameters	Negative PCR ($n=90$)	Positive PCR ($n=10$)	t (*df*)	*P* value
Serum albumin (g/dL)	6.44 ± 0.087	10.49 ± 0.735	− 11.508 (98)	0.00001
AST (U/L)	38.32 ± 1.468	31.70 ± 4.70	1.417 (98)	0.160
ALT (U/L)	52.21 ± 2.029	47.00 ± 6.979	0.801 (98)	0.425

Data are presented as mean ± SE, *P* value ≤ .05 are statistically significant (Independent Samples *T*-test)

Table 3 Results of kidney function in positive and negative *H. canis* PCR testing dogs

Parameters	Negative PCR ($n=90$)	Positive PCR ($n=10$)	t (*df*)	*P* value
Urea (mg/dl)	24.19 ± 0.392	24.00 ± 1.520	0.148 (98)	0.883
Creatinine (mg/dl)	0.895 ± 0.019	0.938 ± 0.076	− 0.673 (98)	0.503

Data are presented as mean ± SE, *P* value ≤ .05 are statistically significant (Independent Samples *T*-test)

3.7 Histopathological finding

Postmortem examination of dead dogs infected with *H. canis* showed hemorrhagic liver and enlargement of the spleen. Moreover, observed sections contained early meronts with their limited number of nuclei. They have exhibited maturing meronts characterized by an increased number of merozoites. The meronts resembled wheel spoke which arranged circularly. Regarding liver samples (Fig. 5d–f), diffuse areas of hepatic hemorrhages with substantial parenchymal loss were observed. The hepatocytes suffered hepatocellular degeneration and necrosis. Also, notice heavily infiltrated mononuclear inflammatory cells in the portal sites.

The microscopic description of the spleen Fig. 5a–c revealed marked lymphoid depletion and a significant expansion of red pulp. The spleen contained enormous amounts of active macrophages. Hemosiderosis was evident with the presence of numerous hemosiderin-laden macrophages. Furthermore, notice the different developmental stages of *H. canis* in the splenic tissue. The monozoic cysts within the host mononuclear cells caused a peripheral dislocation of the host nucleus to the periphery of the cells.

Moreover, observed sections contained early meronts with their limited number of nuclei. They have exhibited maturing meronts characterized by an increased number of merozoites. The characteristic wheel spoke meronts of *Hepatozoon*, the circularly detected peripherally arranged merozoites. Regarding liver samples (Fig. 5d–f), observed diffuse areas of hepatic hemorrhages with substantial parenchymal loss. The hepatocytes suffered hepatocellular degeneration and necrosis. Also, notice heavily infiltrated mononuclear inflammatory cells in the portal sites.

4 Discussion

The protozoan parasite *H. canis* infects dogs by ingesting ticks, which may negatively affect their health and well-being. The present study revealed that all infected cases were infested by *R. sanguineus* only with a 30% infestation rate, and this observation agreed with previous

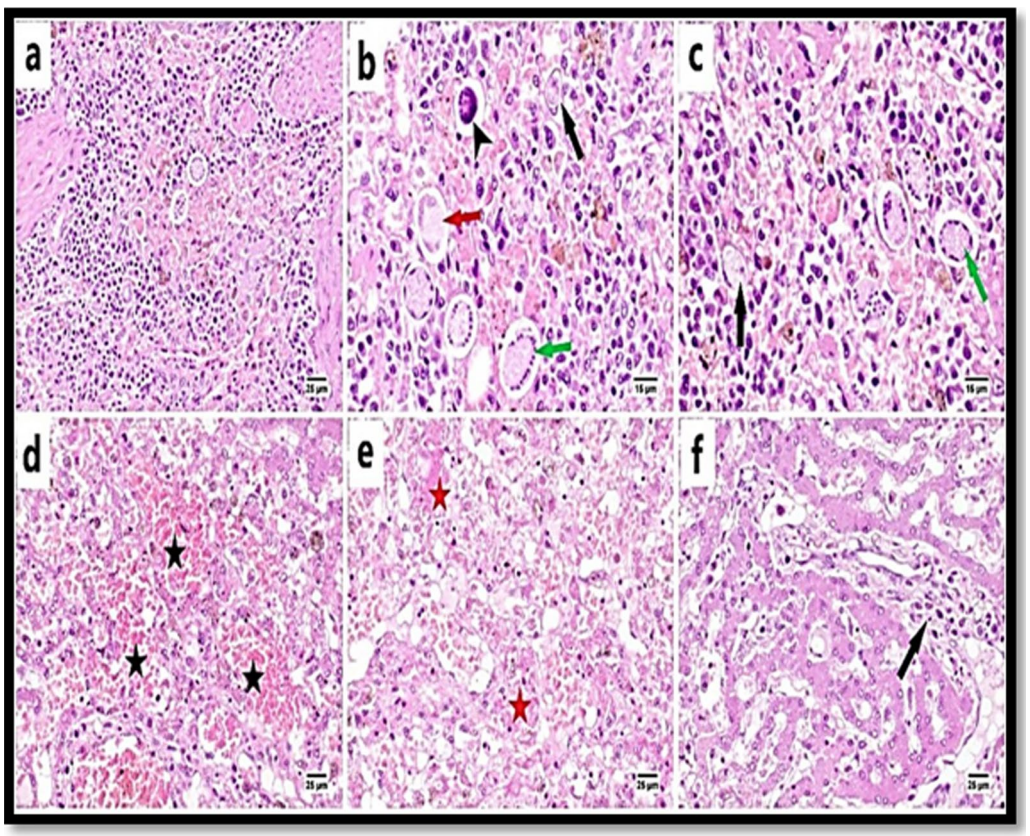

Fig. 5 Photomicrograph of **a–c** spleen and **d–f** liver of dog, H&E-stained, showing **a** the presence of different stages of *H. canis* in splenic tissue, **b** monozoic cyst in the mononuclear cells of the host that contained single cystozoite (black arrow) dislocating the host cell nucleus to the periphery, an early meront with limited number of nuclei (red arrow), maturing meront with increased number of nuclei (arrowhead) and the wheel spoke-shaped meronts (green arrow). **c** Single cystozoite within the host cell (black arrow) and wheel spoke-shaped meront (green arrow); note the presence of golden yellow to brown deposits of hemosiderin pigment. **d** Areas of hemorrhage (stars) within the liver parenchyma, **e** marked hepatocellular necrosis (red stars) and **f** mononuclear inflammatory cells infiltration in the portal area (black arrow) (× 100)

reports by [14, 16, 24, 40, 32]. On the other hand, *H.canis* has also been detected in other tick species, including *Rhipicephalus microplus, Haemaphysalis longicornis* and *Haemaphysalis flava* [48]. *Ixodes ricinus* is widespread in Europe, and *H. canis* DNA was detected in one *I. ricinus* tick collected from the environment in Italy [24], but additional studies suggested that this tick species does not act as a vector for *H. canis* [26]. Transstadial transmission of *H. canis* from *R. sanguineus* larvae to nymphs has been described [27]. Furthermore, the discovery of *H. canis* in additional tick species (Ixodes sp.) offers fresh insights into potential novel vectors for this parasite [38]. They found that *H. canis* DNA was ubiquitous in red foxes, *Ixodes canisuga* nymphs and *I. hexagonus* in Germany.

The prevalence of *H. canis* infection in the current microscopic result was 5%, which is greater than the figures for stray dogs in Cuba obtained by Dáz-Sánchez et al. 19 and Aktas et al. [3] in Turkey (1%). El-Dakhly et al. [21] in Japan, Oliveira et al. [40] in Brazil, and Khalifa and Attia [32] in Egypt recorded that *Hepatozoon* gamont infection in the dog peripheral blood was 23.6%, 8.1% and 30%, respectively. This variation in the prevalence of *H. canis* was caused by various ecological conditions, including the degree of tick manifestation [42].

This study investigated the prevalence of tick-borne pathogens *H. canis* from infested police dogs in Egypt and found that the prevalence was 10% with PCR using the 18S rRNA gene. Previous research recorded that the prevalence of *H. canis* from dogs by PCR was 11.4% in Thailand [31] 30% in India [46], 57.8% in Italy [42], 32.5% in Italy [20], 14% in Italy ([43] and 88.8% in South Africa [39]. In addition, [14] illustrated that 13.5% of domestic dogs were positive for *Hepatozoon* spp. DNA. On the other hand, Aktas et al. [3] in Turkey detected a low occurrence (1%) of *H. canis* infection in dogs.

The findings of the phylogenetic analysis showed that the sequences of *H. canis* that had been obtained clustered with other *H. canis* sequences from carnivores (dogs and foxes) from other nations, which may indicate the presence of *H. canis* strain in necropsied. The findings of the phylogenetic study also demonstrated that the current most prevalent *H. canis* sequence is closely linked to *H. canis* sequences obtained from dogs, confirming the notion that foxes and jackals are essential carriers of this parasite [35].

The partial *H. canis* 18S rRNA gene sequences found in the investigated police dogs in our study are phylogenetically similar to those found in dogs previously (MW362244.1), revealing that 100% nucleotide identity of *H. canis* sequences from different countries in India, Qatar, Portugal, Pakistan, Brazil, Nigeria, Croatia and Brazil with accession numbers in GenBank (KJ605145.1, MF142765.1, LC 018194.1 and KU535868.1). Concerning this, the obtained *H. canis* sequence (MW362245.1) revealed 100.0% nucleotide identity of *H. canis* sequences from dogs in Nigeria and India (JQ976623.1 and MG018464.1).

The sampled police dogs' hematological results revealed a modest (10%) percentage of *H. canis* infection, comparable to the findings on dogs living in Brazilian cities [28, 36]. Hematology and serum biochemistry revealed that the *H. canis*-infected animals did not exhibit significant changes in blood characteristics; however, 5 dogs did have anemia, which is a common finding of *H. canis* infection [8, 28, 51] although it is also a frequent sign for other diseases. Only three dogs showed leucocytosis, a hematological symptom of canine hepatozoonosis, and had the greatest parasitemia (1%) [8, 28, 51]. It may be due to the inflammatory response to tissue invasion and multiplication by *H. canis,* which can be exacerbated by secondary bacterial infections concomitant with other hematozoa.

All animals showed hyperproteinemia, the only change detected by serum biochemistry. However, this finding could be explained by the fact that the dogs also had conditions like *Babesia* spp. or *E. canis* based on Kwon et al. [34]. Infection with *H. canis* led to mild hypoglycemia, mild hyperproteinemia, elevated alkaline phosphatase and decreased blood creatinine, and chloride concentrations in a 2-year-old intact male Maltese dog in Korea. This result concurred with their conclusions.

The *H. canis*-infected stray dogs did not exhibit significant blood serum abnormalities in a laboratory, despite the possibility that they had concurrent illnesses. The absence of clinical symptoms may be related to the relatively low parasitemia of the infected canines (1%). Animals with high parasitemia exhibited a more severe systemic manifestation of the infection than canines with low parasitemia, according to [11].

5 Conclusion

H. canis is one of the series of hemiparasites that cause risk to health and wellness in police dogs. This study registered the sequences of the *H. canis*18S rRNA gene in Egypt for the first time in Genbank (MW362244.1-MW362245.1). Concerning, the results of the Biochemical assay revealed that the parasite has a severe effect on the protein that serum albumin significantly increased in positive PCR testing dogs. According to this study, treating the hemiparasites infecting dogs, eliminating the tick infestation and spraying the neighborhood where the dogs dwell are all advised (ground, dog kennels and wall).

Abbreviations

H. canis	*Hepatozoon canis*
R. sanguineus	*Rhipicephalus sanguineus*
E. canis	*Ehrlichia canis*
EDTA	Ethylenediaminetetraacetic acid
RBCs	Red blood cells count
WBCs	White blood cells count
Hb	Hemoglobin
Hct	Hematocrit
PLT	Blood platelets count
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
18S rRNA	18S ribosomal RNA
H&E	Hematoxylin and eosin
S.E	Standard error

Acknowledgements
We want to acknowledge the staff of the Egyptian Police Academy Veterinary Health Care Unit at K9 that supports the investigation and evaluation of blood analysis of police dogs.

Author contributions
Zaki A.A. collected the samples. Mahdy O.A, Attia M.M and Kahlifa M.M identified the parasites and did the molecular analysis. Al-Mokaddem A.K did the histopathological examination. All authors shared in writing this manuscript and revised it. All authors have read and approved the final manuscript.

Funding
No funding supporting this work.

Availability of data and materials
All the authors declare that all the data supporting the results reported in our article were found included in this article only.

Declarations

Ethical approval and consent to participate
This study was approved and followed the guide of the Ethical committee of the Cairo University, Faculty of Veterinary Medicine; these experiments were performed in compliance with the ARRIVE guidelines.

Consent to publish
Not applicable.

Human and animal resources
I declare that the collection of samples from animals was conducted per local Ethical Committee laws and regulations regarding the care and use of laboratory animals.

Competing interests
No competing of interest in authors.

Received: 23 November 2023 Accepted: 26 March 2024
Published online: 02 May 2024

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