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## Epidemiological and molecular diagnosis of *Ehrlichia canis* infection among dogs

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### ABSTRACT

*Ehrlichia canis* (*E. canis*) is a tick-borne disease, affect dogs in different areas. Traditional diagnostic techniques including hematology, cytology and isolation are valuable diagnostic tools for *E. canis*, however a definitive diagnosis of *E. canis* infection requires molecular investigation techniques. An epidemiological study of *E. canis* infection among dogs in Egypt was carried out using microscopic examination and molecular tools techniques. A total of 170 canine blood samples were collected from different breed of dogs admitted to veterinary clinics in three governorates (Cairo, Giza and Qalyubia). Microscopic examination revealed that 25 out of 170 (14.7%) was positive for *E. canis*. Molecular screening by polymerase chain reaction (PCR) was performed using genus-specific primers followed by PCR using *E. canis* species-specific primers. PCR assay can be detected 27 out of 170 dogs for *E. canis* from examined animals. These results emphasize that serological and molecular studies are needed to clarify the epidemiological feature of the infection in different governorates of Egypt.

## 1. INTRODUCTION

Ehrlichiosis is an important tick-borne infectious disease of dogs and other canids, with higher frequency in tropical and subtropical regions. Ehrlichiosis is caused by *Ehrlichia canis* (*E. canis*), obligatory gram-negative bacteria which infect monocytes, granulocytes and platelets (Mohammadi et al., 2011, Pérez-Macchi et al., 2019). The organism is transmitted by various species of ticks such as the brown dog tick, *Rhipicephalus sanguineus* which is endemic worldwide (Paulino et al., 2018).

*E. canis* have been reported among dogs in different areas all over the world such as North America, Europe, Middle East, North and South Africa. However, the information on the prevalence of *E. canis* among dogs are very limited (Dantas-Torres et al., 2018). Salib and Farhali (2015) reported a percent of 3.5% of dogs in Giza governorate, Egypt were infected as detected by microscopic examination.

*E. canis* characterized by a wide variety of clinical signs like depression, weight loss, anorexia, pyrexia, lymphadenopathy, splenomegaly and a tendency to hemorrhage. Moreover, Diarrhea and vomiting have also been reported (Harrus and Waner, 2011).

Infection with *E. canis* associated with hematological abnormalities include thrombocytopenia, mild anemia and mild leucopenia during the acute form of the disease (Heeb et al., 2003).

Traditional diagnostic methods such as microscopic smear, serology and isolation are reliable method for detection of *E. canis*. Microscopic examination use to detect morulae in blood smear to confirm the presence of *E. canis* but the efficacy of this technique is very limited especially in low parasitemia clinical cases (Øines et al., 2010). The serological detection of anti- *E. canis* antibodies may be performed by Indirect Fluorescent Antibody Test (IFAT) or Dot-ELISA (Cadman et al., 1994). IFAT is the serological assay most widely used for the diagnosis of canine ehrlichiosis (Harrus and Waner, 2011).

Furthermore, the antibodies against *E. canis* are usually absent early during the first two weeks of infections and can be cross react with several other Ehrlichial organisms (Filipović et al., 2018). On the other hand, PCR and sequencing are sensitive methods for detecting and characterizing *E. canis* DNA. Detection of *E. canis* DNA can be achieved as early as 4–10 days post-inoculation (Bunroddith et al., 2018). Several assays are based on different target genes (e.g. *16S rRNA*, *p28*, *p30*, *dsb*, *VirB9*) however the *16S rRNA* and the *p30*-based PCR assays are most commonly used. This technique may be useful for the discrimination between different Ehrlichial species and strains. There are several advantages for PCR (either conventional or real time) over serology, namely, (1) the early detection of DNA before seroconversion occurs, (2) its higher sensitivity, and (3) the detection of Ehrlichial DNA rather than anti-*E. canis* antibodies, probably indicating active infection rather than exposure (Harrus and Waner,

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2011, Pérez-Macchi et al., 2019). So, the aim of this study for determining the prevalence of Canine monocytic ehrlichiosis (CME) among dogs in Egyptian localities, and molecular detection of *E. canis* infection in dogs.

## 2. MATERIAL AND METHODS

### 2.1. Ethic statement

Samples collection was performed under owner's consent, and the study was approved by the Internal Ethics Review Committee of Faculty of Veterinary Medicine, Benha University.

### 2.2. Samples collection and preparation

A total 170 blood samples were collected from different breeds of dogs which were adapted to veterinary pet clinics in Cairo, Giza and Qalyubia during 2018. The blood samples were collected on EDTA from saphenous or cephalic vein of dogs showed clinical manifestations suspected to be for Ehrlichiosis. The epidemiological and clinical data of each examined dogs were obtained from owner include sex, breed and age

### 2.3. Direct Microscopic smear

Blood smears were prepared from collected blood samples of all dogs under investigation and stained by Giemsa stain as previously described by Rathore and Sengar (2005) to detect *E. canis* morulae in monocytes.

### 2.4. Molecular detection of *E. canis*

DNA was extracted from whole blood of examined dogs using QIAamp® DNA Mini (QIAGEN GmbH, Hilden, Germany) according to manufacturer's instructions. The extracted DNA was stored at -20 °C until being used in PCR assay.

PCR assay targeting 16S rRNA gene of *E. canis* using specific pairs of primers was carried out on all blood samples to detect the presence of *E. canis* (Inokuma et al., 2001).

forward CANIS 5'-CAA-TTA-TTT-ATA-GCC-TCT-GGC-TAT-AGG-A-3'
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reverse GAIUR 5'-GAG-TTT-GCC-GGG-ACT-TCT-TCT-3'
---

PCR reaction was carried out in a 25 µl reaction volume. The PCR master mix contained 1 µl of each primer (10 pmol/µl), 12.5 µl of Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, US), and 5.5 µl of RNase-free water. Finally, 5.0 µl of DNA template was added. The thermal cycling procedure was; 1 cycle of 5 min at 95 °C, 40 cycles of 30 Sec at 95 °C, 30 Sec at 62 °C, 1 min at 72 °C, and final cycle of 5 min at 72 °C. The amplified PCR products were visualized at 1.5% agarose gel.

### 2.5. Statistical analysis

The obtained data was analyzed using SPSS V17 and chi-square test to determine the relation of different variables and infection rate of the disease at different variables. The results were considered significant at a probability level  $\leq 0.05$ .

## 3. RESULTS

The results of microscopic examination of stained blood smears revealed detection of some inclusion bodies in the nucleated cells and morulae noted in monocytes as in figure 1. The result showed 25 dogs out of 170 were positive. The values of infection rate of *E. canis* was in non-significant ( $p=0.263$ ) difference for the three localities under the study and the rate was higher in Giza (23.3%) governorate in comparison with Cairo (14.4%) and Qalyubia (10%) as in table (1).

The obtained results for detection of ehrlichiosis in varied breeds revealed that the *E. canis* can infect all breeds of dogs. The infection rate was higher in German breed (20%) in comparison with other examined breeds as in table 2. The PCR amplification using species specific primers revealed that 27 out of 170 (15.8%) of examined dogs were positive for *E. canis*, with 409 bp amplified amplicon as shown in Fig 1.

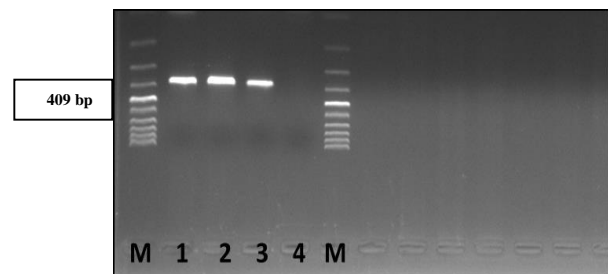


Fig1 Amplification of the 16S rRNA gene from DNA of some examined dogs, approximately 409 bp. M; marker, Lane 1-3 positive samples (409bp) and lane 4 negative sample

Table 1 Prevalence of *E. canis* in different study areas

Sample site	Total number of samples	No of positive (%)	P value
Qalyubia	50	5 (10%)	0.263 <sup>NS</sup>
Cairo	90	13 (14.4%)	
Giza	30	7 (23.3%)	
Total	170	25 (14.7%)	

The result is non-significant at  $p > 0.05$ .

Table 2 Breed prevalence of *E. canis* in blood samples

Breed	Number of samples	No of positive (%)	P value
German	70	14 (20%)	0.490 <sup>NS</sup>
Boxer	10	1 (10%)	
Rottweiler	30	3 (10%)	
Lolo	10	1 (10%)	
Golden retriever	20	1 (5%)	
Pit bull	30	5 (16.6%)	
Total	170	25 (14.7%)	

The result is non-significant at  $p > 0.05$ .

## 4. DISCUSSION

CME is a tick-borne disease of increasing importance in dogs and has become an active area of research in recent year.

*E. canis* has been detected and reported in dogs from many parts of the world (Harrus and Waner, 2011; Rani et al., 2011). The obtained results revealed a 14.7% overall infection rate of *E. canis* (N = 25) on basis of blood samples from the three districts, suggesting that *E. canis* is prevalent in Giza province but the values of infection rate of *E. canis* was in a non-significant difference in different localities indicating that geographical features and climatic conditions do not affect the disease.

These obtained results were come in accordance with that recorded previously (Guedes et al., 2015), who reported the prevalence rate of *E. canis* by microscopic examination was 12.4% in dogs while Salib and Farghali (2015) reported lower rate for *E. canis* in previous study in dog from Egypt, it was 3.5%.

The molecular diagnosis of *E. canis* among dogs revealed that 27 out of 170 positives for *E. canis*. The results confirmed that PCR was more sensitive and overcome the limitation of microscopic examinations (Bunroddith et al., 2018).

## 5. CONCLUSION

In conclusion, the present study confirms presence of *E. canis* among dogs in Egypt and epidemiological surveying of the disease in other localities should be applied to plan control measures of the disease among dogs. Further studies are needed for molecular typing and phylogenetic analysis of the pathogen.

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