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Advanced Studies on Tick Borne Blood Parasites Among Pets (Dogs)

A thesis Presented by

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Abstract

The present study screened house hold and kenneled dogs with its attached ticks for tick-borne pathogens (TBPs) by traditional and molecular techniques. Blood samples were collected from 208 dogs from sexes, different ages and breeds in Cairo and Giza governorates during period between March 2018 to February 2019. Additionally, 1386 ticks were collected from 144 infested dogs then, divided to 546 ticks were dissected for preparation of hemolymph, mid gut and salivary gland smears, 120 female ticks were kept in lab till egg laying for preparation of 360 egg smears and 720 engorged ticks were used for preparation of 144 tick pools for PCR. Results showed that, all ticks collected in the present study were identified as *Rhipicephalus sanguineus*. TBPs were detected microscopically in 35.58% (74/208) of examined blood samples including, *Theileria equi* in 25/208 (12.02%) dogs followed by *Anaplasma* and *Ehrlichia* in 23/208 (11.1%) finally, *Babesia canis* in 17/208 (8.2%). While *Hepatozoon canis* was not detected microscopically in blood smears. Co-infections were observed in 9/208 (4.33%). The total prevalence rates of infection with TBPs in ticks were 44.69%, 68.50% and 15.75% in hemolymph, mid gut and salivary gland respectively. Significant difference in total TBPs rate of infection between different seasons and age groups with P value < 0.05. While breed and sex have no significant effect on rate of infection. Ultrastructure of different TBPs stages were described in details within mid gut and salivary gland of *R. sanguineus* by TEM as, the early oocyst and sporocysts of *H. canis, A. phagocytophilum* colony , penetrating kinetes and sporozoites (Infective stages) of *Theileria equi* and *Babesia canis*. PCR amplified a monomorphic DNA fragment of 560 bp size in case of *Babesia* and *Theileria* spp, 670 bp in case of *H. canis* and 450 bp in case of *Anaplasma* and *Ehrlichia* spp. Overall molecular prevalence rate of TBPs was 51.61% and 36.8% of examined dog's blood and ticks. *Theileria* spp. recorded the highest prevalence rate in (25.81% and 10.42%) dogs followed by, *Anaplasma* and *Ehrlichia* in (19.35% and 20.83%), then *Babesia canis* in (6.45% and 5.55%). While, *H. canis* recorded the lowest prevalence rate (0% and 2.8%) in examined blood and Tick respectively. Sequence analysis identified seven different species of TBPs, namely *Theileria equi, B. canis vogeli, H. canis, E. canis, A. phagocytophilum, A. marginale* and *A. Platys*. The identified TBPs were accessed on the GenBank under accession number: MT533853, MT533854, MT533857, MW237710, MW237711 and MT533884 for *Theileria equi* isolates, MW432533 for *B. canis*, MZ203845 for *H. canis*. While, *Anaplasmataceae* family accession numbers were MZ068099 and MZ203829 for *A. platys* and *A. phagocytophilium* respectively, MZ203830, MZ203832, MZ203831 and MZ203834 for A. marginale. MZ191504, MZ191505 and MW433608 for *E. canis* from dog's blood and ticks (*R. sanguineus*).

Keywords: *Anaplasma*, *Babesia*, *Hepatozoon*, PCR, *Rhipicephalus sanguineus*, *Theileria*, TEM, TBPs.

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List of Abbreviations

Abbreviations	Description
ME	Microscopic Examination
PCR	Polymerase Chain Reaction
TBPs	Tick-Borne Pathogens
TBDs	Tick-Borne Diseases
EDTA	Ethylene Diamine Tetra Acetic Acid
PH	Degree of acidity and alkalinity
TBE buffer	Tris borate ethylene diamine tetra acetic acid buffer
µl	Micro liter
%	Percent
DNA	Deoxy Ribo Nuclic Acid
RPM	Revelution Per Minute
µm	Micrometer
χ^2	Pearson chi-square
TEM	Transmission Electron Microscope
bp	Base Pair
ELISA	Enzyme Linked Immune Sorbent Assay
IFAT	Indirect Flourcent Antibody Tichnique
16S-rRNA	16 Small Ribosomal Ribo Nuclic Acid
ssu-rRNA	Small SubUnite Ribosomal Ribo Nuclic Acid
18S-rRNA	18Small Ribosomal Ribo Nuclic Acid