

Coinfections of *Leishmania infantum* and other zoonotic pathogens in apparently healthy Cirneco dell'Etna dogs' breed. Screening of hematological traits

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Background

Cirneco dell'Etna (CE) (Figure 1), is an autochthonous canine breed of Sicily (IT), the only Italian breed recognized in section 7 of Group 5: Spitz and primitive types of FCI [1] like Ibizan hound and Canarian Warren hound. The immune response to *Leishmania* infection has been studied in Ibizan hound, which presents certain resistance to infection [2], probably because of genetic variants found in genes encoding cytokines related to immune response [3]. Although both breeds are phylogenetically close [4], no study has been carried out regarding the susceptibility of CE dogs to *Leishmania* or other infections endemics of the Mediterranean basin. This study analyzes the occurrence of *Leishmania* infection, coinfections, and hematological and biochemical parameters of CE without clinical signs infected and not infected by *L. infantum* and other pathogens.



Figure 1: Picture of Cirneco dell'Etna male dog.

Material and methods

Twenty-three CE adult dogs (5 males and 18 females) ranging from 13 to 145 months of age, apparently healthy based on a physical examination, breed in the same geographic area and management, were tested. For all animals, epidemiological data were collected. Serological testing was performed for *Leishmania infantum*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Rickettsia rickettsii* by quantitative IFAT method, and hematological and biochemical parameters analysis was performed. Statistical analysis was performed by ANOVA test or Kruskal-Wallis test for normally distributed or non-normal distributed data, respectively. The statistical analysis was performed using R software and p -value < 0.05 was considered statistically significant.

Results

The frequency of *L. infantum*, *A. phagocytophilum*, *R. rickettsii* were similar, and higher than *E. canis* infection (Figure 2). Among *Leishmania* spp. seropositive animals, three out of six presented coinfections with *R. rickettsii*. Only one dog seronegative and one seropositive for *Leishmania* spp. presented coinfections with *E. canis* and *A. phagocytophilum*. Regarding biochemical and hematological features, no differences were found between *L. infantum* seropositive and seronegative CE dogs, whereas other pathogen infections modified different parameters. Biochemical and hematological data recovered for *L. infantum* seronegative and seropositive dogs coinfecting with other pathogens are shown in Tables 1 and 2 respectively (bold type indicates statistical differences).

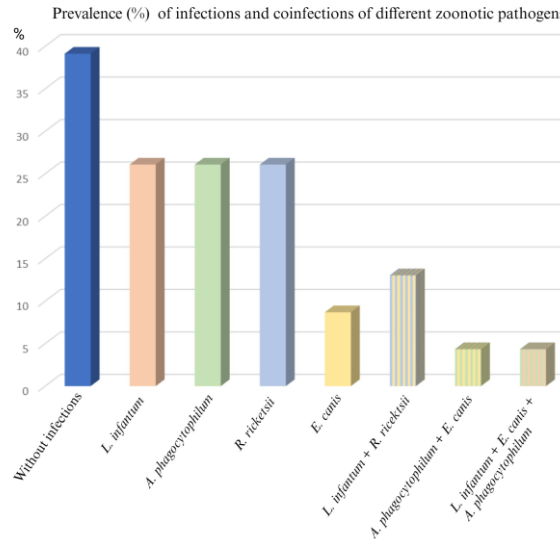


Figure 2: *Leishmania infantum*, *Anaplasma phagocytophilum*, *Rickettsia rickettsii*, *Ehrlichia canis* and coinfections in Cirneco dell'Etna dogs (percentages amongst 24 dogs tested).

Table 1: Biochemical and hematological parameters of *Leishmania* spp. seronegative without or with other infections.

	Pathogen infection				
	RI	Mean ± SD	None	<i>A. phago.</i>	<i>R. rickettsii</i>
Complete blood count					
RBC (10⁶/μL)	5.1-7.6	7.1±0.7	7.3±0.7	7.0±0.7	7.3±4.6
Hemoglobin (g/dL)	12.4-19.2	16.7±1.6	16.9±1.9	16.1±1.3	17.3±1.3
Hematocrit (%)	35-52	51.2±4.4	52.1±4.6	48.6±3.2	53.7±4.2
MCV (fL)	60-77	71.8±3.3	72.0±3.5	69.7±3.2	74.1±2.4
MCH (pg)	21.9-26.3	23.4±0.8	23.3±0.8	23.1±0.7	23.9±1.0
MCHC (g/dL)	34.4-38.1	32.7±1.3	32.5±0.8	33.1±0.8	32.53±0.4
WBC (10⁹/L)	5.6-20.4	9.8±3.5	11.0±4.5	8.4±0.9	7.8±1.5
Neutrophils (10⁹/L)	2.9-13.6	6.4±3.2	7.3±4.1	5.3±0.7	5.0±1.3
Lymphocytes (10⁹/L)	1.1-5.3	2.3±0.5	2.5±0.5	2.4±0.6	1.9±0.6
Monocytes (10⁹/L)	0.4-1.6	0.8±1.9	0.4±0.2	0.4±0.1	3.0±0.6
Platelets (K/μL)	150-500	246.6±104.9	260.9±103.4	342.5±75.3	135.7±36.4
PCT (%)	0.1-0.5	0.2±0.1	0.2±0.1	0.3±0.0	0.08±0.05
Biochemical parameters					
Total protein (g/L)	54-71	59.4±2.6	59.8±2.7	59.7±3.4	58.3±2.3
Albumin (g/L)	26-33	34.1±0.2	34.4±1.7	33.0±2.4	34.0±1.7
A/G ratio	0.86-1.93	1.4±0.2	1.4±0.2	1.3±0.3	1.4±0.3
Creatinine (mg/dL)	0.5-1.5	0.9±0.1	0.9±0.1	0.9±0.1	1.0±0.2
Urea (mg/dL)	21.4-59.9	12.4±2.8	12.3±2.5	11.3±1.0	15.0±4.4
GOT (U/L)	0-90	57.7±25.9	53.4±19.4	38.2±6.2	85.0±36.9
Glucose (mmol/L)	60-120	71.5±7.4	71.9±7.5	77.8±5.2	64.7±1.5
Serum Protein Electrophoresis					
Alpha-1 globulin (g/L)	1.7-4.5	3.8±0.5	4.0±0.5	3.8±0.5	33.3±0.6
Alpha-2 globulin (g/L)	3.8-10.2	5.3±1.2	5.7±1.3	5.0±1.4	46.7±0.6
Beta-1 globulin (g/L)	2.9-10.7	4.9±1.2	5.0±0.9	5.5±1.7	4.0±1.0
Beta-2 globulin (g/L)	2.17-5.81	6.5±1.9	6.2±1.8	6.8±1.3	7.3±3.2
Gamma globulin (g/L)	2.6-11.7	4.3±1.0	4.1±1.1	5.3±0.9	4.0±0.0

RI: Reference interval. SD: standard deviation.

Table 2: Biochemical and hematological parameters of *Leishmania* spp. seronegative without or with other infections.

	RI	Mean + SD	Pathogen infection	
			<i>L. infantum</i>	<i>L. infantum</i> + <i>R. rickettsii</i>
Complete blood count (CBC)				
RBC (10 ⁶ /μL)	5.1-7.6	7.3±0.8	6.7±0.4	7.9±0.7
Haemoglobin (g/dL)	12.4-19.2	16.7±2.0	15.6±0.5	18.2±1.4
Haematocrit (%)	35-52	51.6±7.4	45.9±2.1	57.8±1.4
MCV (fL)	60-77	70.8±3.6	68.8±1.2	73.8±1.5
MCH (pg)	21.9-26.3	23.0±0.9	23.3±0.7	23.2±0.5
MCHC (g/dL)	34.4-38.1	32.1±1.2	33.9±0.5	31.5±0.4
WBC (10 ⁹ /L)	5.6-20.4	9.0±1.1	9.9±0.5	8.5±1.2
Neutrophils (10 ⁹ /L)	2.9-13.6	5.3±1.1	5.1±0.0	5.9±0.8
Lymphocytes (10 ⁹ /L)	1.1-5.3	2.8±1.3	3.1±0.9	1.7±0
Monocytes (10 ⁹ /L)	0.4-1.6	0.3±0.2	0.3±0.4	0.3±0.1
Platelets (K/μL)	150-500	205.3±201.3	511.5±258.1	107.7±55.0
PCT (%)	0.1-0.5	0.14±0.12	0.3±0.02	0.07±0.06
Biochemical parameters				
Total protein (g/L)	54-71	61.0±3.0	59.0±3.5	60.7±2.5
Albumin (g/L)	26-33	31.5±4.6	31.5±0.5	34.0±1.7
A/G ratio	0.86-1.93	1.1±0.4	1.2±0.6	1.3±0.2
Creatinine (mg/dL)	0.5-1.5	0.9±0.09	0.8±0.0	1.0±0.1
Urea (mg/dL)	21.4-59.9	11.0±0.6	11.5±0.7	10.7±0.6
GOT (U/L)	0-90	71.4±39.9	36.5±0.7	89.0±42.1
Glucose (mmol/L)	60-120	67.4±9.3	81.5±9.2	62.2±2.0
Serum Protein Electrophoresis				
Alpha-1 globulin	1.7-4.5	4.0±0.9	4.5±0.7	4.0±1.0
Alpha-2 globulin	3.8-10.2	6.2±1.2	6.5±2.1	6.0±1.0
Beta-1 globulin	2.9-10.7	6.2±1.6	6.0±2.8	6.3±1.5
Beta-2 globulin	2.17-5.81	6.5±1.2	6.5±2.1	6.3±1.2
Gamma globulin	2.6-11.7	6.7±6.2	5.0±1.4	3.7±1.2

RI: Reference interval. SD: standard deviation.

Conclusions

The occurrence of *L. infantum* infection in CE dogs was lower than data previously published in this region for other canine breeds, which have been estimated at around 40% [5]. In this canine breed, the *L. infantum* infection did not modify serum levels among parameters analyzed, whereas coinfections with other zoonotic pathogens changed levels of different biochemical and hematological parameters, such as red blood count (RBC), haemoglobin, haematocrit, platelets, and lymphocytes count, among others, whereas parameters of serum electrophoresis remained unchanged. These results could indicate a certain resistance to *L. infantum* infection in this canine breed native to the Mediterranean region. More studies are necessary to determine the immune response against infection by *Leishmania* spp. and other pathogens endemic to the Mediterranean region in this breed native to Sicily.

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