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Seroprevalence of zoonotic pathogens and related haematological and biochemical profiles in Fonni's dogs in rural conditions

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ABSTRACT

Fonni's dog is a canine breed autochthonous to the Mediterranean area, specifically from Sardinia (Italy). A total of 190 dogs were examined to determine the seroprevalence of different pathogens endemic in this region and related haematological profile. The seropositivity of pathogens was 65.0 %, 41.3 %, 28.7 %, and 18.9 % for *Rickettsia* spp., *Ehrlichia canis*, *Anaplasma. phagocytophilum*, and *Bartonella* spp., respectively. *Leishmania infantum* and *Toxoplasma gondii* presented the same seroprevalence (5.6 %) respectively. The number of pathogens in co-seropositivity did not change the haematological parameters evaluated, whereas the age had an effect on several of them, including albumin (ALB), alkaline phosphatase (ALP), calcium (CAL), total cholesterol (COL), creatine phosphokinase (CPK), creatinine (CRE), aspartate aminotransferase (GOT), lipase (LIP), phosphorus (P), total protein (PRO) and triglycerides (TRI). Pathogens evaluated influenced different parameters. Specifically, *Rickettsia* spp. decreased CPK activity, creatine and glucose levels and increased phosphorus. *T. gondii* increased CPK activity and decreased glucose levels, and *E. canis* decreased gamma glutamyl transferase (GGT) activity. Finally, *L. infantum* seropositivity decreased CPK and increased GOT activities. The results observed in Fonni's dogs related to seroprevalence of *L. infantum* and associated haematological parameters indicate that this canine breed could exhibit different behaviour from that of other canine breeds when faced with this pathogen. Further studies are necessary to elucidate the cause of these differences.

1. Introduction

Canine vector-borne diseases (CVBDs) is a significant challenge not only for veterinarians but also for humans, as many are zoonotic. CVBDs are caused by bacteria, parasites, and other pathogens, and are transmitted by arthropods such as ticks, phlebotomine sand flies, mosquitoes and fleas. The spread of these vectors has increased in recent years due to rising global temperatures, leading to the detection of infected dogs in non-endemic regions (Otranto et al., 2009). Some of the most common CVBD include rickettsiosis, ehrlichiosis, anaplasmosis, bartonellosis and leishmaniosis (Otranto and Dantas-Torres, 2010). These zoonoses are prevalent in the Mediterranean basin, where there are different native canine breeds. Differences in immune response to infections have been observed in certain breeds, particularly those from regions where the pathogens are endemic. For example, this phenomenon has been observed in cattle breeds resistant to trypanosome infections (Paguem et al., 2020) and in Sicilian small ruminants resistant to spongiform encephalopathy (Vitale et al., 2016).

Among dogs, the Ibizan hound, native to Spain's Mediterranean region, appears resistant to leishmaniosis, exhibiting a different immune response compared to other breeds (Álvarez et al., 2022). Another Mediterranean breed, the Cirneco dell'Etna from Sicily, also shows a different immune response to this infection (Amato et al., 2024; Martínez-Sáez et al., 2024). However, research on other canine breeds and pathogens remains limited.

One of the oldest Mediterranean breeds is the Sardinian Sheperd, or Fonni's Dog, believed to have originated before the 19th century (Sechi et al., 2017). This canine breed, native to Sardinia, is primarily used for

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livestock guarding and hunting hares and boars. These dogs typically live outdoors and are fed and diet mainly consisting of raw meat (Cortellari et al., 2023; Cocco et al., 2024a). The breed is classified by the Italian Kennel Club (ENCI) under Group 2: Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs, 2: Molossian type, and is currently admitted to the RSA (Open Supplementary Register) with recognition procedures underway due to its restricted breed status.

Understanding haematological and biochemical variations in canine breeds exposed to vector-borne pathogens can provide insights into the mechanisms of resistance to infection by these pathogens. This study aims to investigate the seroprevalence of different zoonotic pathogens and associated biochemical and haematological parameters in Fonni's dogs raised in rural conditions.

2. Material and methods

2.1. Animals and experimental design

A total of 190 intact Fonni's dog were included in this retrospective study carried out at the Veterinary Teaching Hospital of the University of Sassari (Sardinia, Italy). All animals were bred outdoors on Sardinia Island and had a diet that included raw meat, as the traditional breeding system. The inclusion and exclusion criteria of all animals are shown in Table 1. For all animals included, a biochemical profile was realized in the hospital and epidemiological data of age, sex, status vaccination and clinical signs were recovered. Only dogs without clinical signs were included, and they were grouped as puppy (less than 9 months), young (between 9 and 24 months), adults (between 2 and 8 years old) and senior (8 or more years old), following the recommendations of FCI (FCI,). Five milliliters of whole blood were taken by jugular venipuncture with an anticoagulant tube. The tubes were centrifugated at 5000 g to obtain serum samples. The serum samples were stored at 20ºC during two months until use to preserve haematological and biochemical values.

2.2. Parasitic and biochemical parameters

Serum samples were tested for *Anaplasma phagocytophilum*, *Bartonella* spp., *Ehrlichia canis*, *Leishmania infantum*, *Rickettsia* spp., and *Toxoplasma gondii*. Antibodies to the pathogens were measured by indirect immunofluorescence test (IFA) with the following protocols. For *A. phagocytophilum*, a commercial kit with slides containing antigen were used (*Anaplasma phagocytophilum* IFA canine IgG antibody kit, Fuller laboratories, California, USA). According to manufacturer's instructions, serum titres higher than 1:80 were considered positive. IgG and IgM were detected for *Bartonella* spp. using slides with the antigen *Bartonella* spp. (Houston 1 ATCC 49882) cultured in the L929 fibroblast cell line following the protocol of Neupane et al. (2018) (Neupane et al., 2018). Positivity for IgM was considered an acute infection, whereas positivity for IgG was considered a past non-acute infection. Titres higher than 1:40 were considered positive.

Table 1
Inclusion and exclusion criteria for animals included in the study.

Inclusion criteria	Exclusion criteria
Clinically healthy upon physical examination	Physical or historical signs of any kind
Free from internal and external parasites	Any pharmacological treatment, including ectoparasite treatment in the month prior to blood sampling
Good nutritional condition	History of vector-borne disease
No use of internal and external antiparasitic	Vaccinate against the investigated disease
Outdoor dogs	Previously treated for any pathogen evaluated
Owner provided informed consent	
Not previously treated for any pathogen evaluated	

To detect anti-*E. canis* antibodies, the protocol described by Dawson et al. (1991) (Dawson et al., 1991) was followed and the anti-*L. infantum* IgG antibodies were detected using in-house IFAT following the laboratory procedures of OMS Manual of Diagnostic Tests and Vaccines of Terrestial Animals (Manual of diagnostic tests and vaccines for terrestrial animals, 2021), considering titres of at least 1:80 as positives for two pathogens.

A commercial canine *Rickettsia* spp. IgG Indirect Fluorescent Antibody test (IFAT) kit was used to detect anti-*Rickettsia* spp. antibodies, and the positivity threshold was established at least 1:128, following the manufacturer recommendations. Finally, anti-*T. gondii* antibodies IgG and IgM were detected through indirect immunofluorescence (IFI), considering IgG positive titre $\geq$ 640, IgM positive titre $\geq$ 160, and with cut-off titres for both, 80.

For *Bartonella* spp. and *T. gondii* active infection was considered if the result was positive for IgM, whereas positive for IgG was considered past non-active infection.

Biochemical profile was carried out with a commercial kit following the manufacturer's instructions, and an automated UV Spectrophotometer (SEAC, Slim, Florence, Italy), and included albumin (ALB), alkaline phosphatase (ALP), amylase (AM), total Bilirubin (BT), calcium (CAL), total cholesterol (COL), creatine phosphokinase (CPK), creatinine (CRE), gamma glutamyl transferase (GGT), glucose (GLU), aspartate aminotransferase (GOT), glutamate-pyruvate transaminase (GPT), lipoprotein lipase (LIP), phosphorus (P), total protein (PRO), triglycerides (TRI), and urea (UR).

Biochemical parameters were considered altered when they were outside the reference intervals, and serum protein electrophoretic patterns were defined in accordance with published guidelines (Corrigan, 2011).

2.3. Statistical analysis

Normality and homoscedasticity of quantitative data were checked by Shapiro-Wilk and Levene test, respectively. Biochemical parameters and epidemiological data were analyzed using the General Linear Model procedure (PROC GLM) of the SAS statistical package (version 9.2, North Carolina State University, USA). The model was carried out with sex, age, number of infections, and seropositivity for the six pathogens evaluated as fixed effects and pairwise interactions were included. A p-value 0.05 was considered statistically significant.

3. Results

Ninety-five dogs were males (50.0 %), and ninety-five were females (50.0 %). The age range was between 2 months to 15 years old, and 25 dogs were classified as puppies (13.2 %), 49 as young (25.8 %), 89 dogs as adults (46.8 %) and 27 as senior (14.2 %).

Among the 190 dogs analyzed, 143 were seropositive for one or more pathogens (75.3 %). Of the 47 seronegative animals, 12.8 % had IgG (indicating past infection) for *Bartonella* spp. (six dogs) and 21.3 % for *T. gondii* (10 dogs). Considering only active infections, the most common presentation was single infection (37.9 %), followed by double infections (27.9 %). The most prevalent pathogen was *Rickettsia* spp. (93 dogs, 65.0 %), followed by *E. canis* (59 dogs, 41.3 %), *A. phagocytophilum* (41 dogs, 28.7 %), *Bartonella* spp. (27 dogs, 18.9 %), and both *L. infantum* and *T. gondii* (8 dogs, 5.6 % for both infections). A similar distribution was observed in the 72 dogs with single infection (37.9 %), where *Rickettsia* spp. was the most common (39 dogs, 54.2 %), followed by *E. canis* (13 dogs, 18.1 %), *A. phagocytophilum* (10 dogs, 13.9 %), *Bartonella* spp. (6 dogs, 8.3 %), *T. gondii* (3 dogs, 4.2 %) and *L. infantum* (1 dogs, 1.4 %). Multiple infections were found in 71 dogs, with the most common being a double infection by *E. canis* and *Rickettsia* spp. (20 dogs). None of the dogs in this study presented multiple infections involving all the pathogens analyzed (Table 2).

The most relevant epidemiological factor was age, which affected

Table 2
The number and percentage of animals infected with different pathogens analyzed.

Number of infections	Infective pathogen	Number of animals (%)	Percentage of total
None pathogen		47	24.74
One pathogen		72 (100)	37.89
	A. phagocytophilum	10 (1.39)	5.26
	Bartonella spp.	6 (8.33)	3.16
	E. canis	13 (18.06)	6.84
	L. infantum	1 (1.39)	0.52
	Rickettsia spp.	39 (54.17)	20.53
	T. gondii	3 (4.17)	1.58
Two pathogens		53 (100)	27.89
	A. phagocytophilum + *Bartonella* spp.	3 (5.66)	1.58
	A. phagocytophilum + *E. canis*	5 (9.43)	2.63
	A. phagocytophilum + *Rickettsia* spp.	10 (18.87)	5.26
	Bartonella spp.+ *E. canis*	3 (5.66)	1.58
	Bartonella spp.+ *Rickettsia* spp.	4 (7.55)	2.11
	E. canis + *L. infantum*	2 (3.77)	1.05
	E. canis + *Rickettsia* spp.	20 (37.77)	10.53
	L. infantum + *Rickettsia* spp.	3 (5.66)	1.58
	R. rickettsii + *T. gondii*	3 (5.66)	1.58
Three pathogens		16 (100)	8.42
	A. phagocytophilum + *Bartonella* spp.+ *E. canis*	4 (25.00)	2.11
	A. phagocytophilum + *E. canis* + *Rickettsia* spp.	5 (31.25)	2.63
	A. phagocytophilum + *L. infantum* + *Rickettsia* spp.	1 (6.25)	0.53
	A. phagocytophilum + *Rickettsia* spp.+ *T. gondii*	1 (6.25)	0.53
	Bartonella spp.+ *E. canis* + *Rickettsia* spp.	5 (31.25)	2.63
Four pathogens	*A. phagocytophilum* + *Bartonella* spp.+ *E. canis* + *Rickettsia* spp.	1 (100)	0.53
Five pathogens	*A. phagocytophilum* + *Bartonella* spp.+ *E. canis* + *L. infantum* + *Rickettsia* spp.	1 (100)	0.53

several biochemical parameters, including ALB, ALP, CAL, COL, CPK, CRE, GOT, LIP, P, PRO, and TRI. ALP, CAL, CPK, and P were higher in puppies than in other age groups, whereas COL was higher in both puppies and seniors compared to young and adult dogs. ALB was higher in young and adult dogs, while LIP, PRO and TRI increased with age. Conversely, GOT decreased with age (Fig. 1). Sex affected GPT levels, which higher in males (63.3 ± 29.7 units/L) than in females (52.9 ± 27.0 units/L). The number of infections did not affect any of the biochemical parameters evaluated (Table 3). On the contrary, the infective pathogen altered several parameters, including CPK, CRE, GGT, GLU, GOT and P. In Specifically, *Rickettsia* spp. infection decreased CPK, CRE and GLU serum levels, and increased P levels (Fig. 2). *L. infantum* infection also decreased CPK values and increased GOT serum levels (Fig. 3). *T. gondii* increased CPK and decreased GLU levels (Fig. 4), while *E canis* infection decreased GGT serum levels, which were 9.5 ± 3.5 and 11.0 ± 2.8 units/L in infected and non-infected dogs, respectively.

4. Discussion

This study summarised the prevalence of various biochemical parameters and the differences observed between infected and non-infected Fonni's dogs. The results indicated a high seroprevalence of *Rickettsia* spp. (54.2 %), followed by *E. canis* (41.3 %), with co-seroprovalence for both pathogens (*Rickettsia* spp. and *E. canis*) found in 10.5 % of total population. The primary factor influencing biochemical parameters was age, while the number of infections had no significant effect. The infective pathogen influenced several biochemical parameters, with the most frequent change being a decrease in CPK levels in *Rickettsia* spp. and *L. infantum* seropositive dogs, and an increase in *T. gondii* seropositive dogs. Additionally, *Rickettsia* spp. seropositivity was associated with decreased CRE and GLU levels, while *T. gondii* also reduced GLU levels. In contrast, *Rickettsia* spp. and *L. infantum* increased P and GOT levels, respectively. *E. canis* reduced GGT levels, while other pathogens did not significantly alter biochemical values.

The seropositivity rates for *Rickettsia* spp., *E. canis* and *A. phagocytophylum* were consistent with previous studies conducted in the Italian islands (Mendoza-Roldan et al., 2021) and other Mediterranean countries (Laušević et al., 2019; Sgroi et al., 2022). However, limited research existed on *Bartonella* spp. seroprevalence, although Belkhiria *et al.* (2017) reported a rate of 19.5 % in Tunisia (Belkhiria et al., 2017).

In contrast, the *L. infantum* seroprevalence in this study (12.5 %) was lower than was previously reported in Portugal and Sardinia [2021]. This discrepancy could be attributed to the specific breed used in this study. Fonni's dog are large (weighing more than 25 kg) and native to Sardinia, where leishmaniosis is endemic. Rombola *et al.* (2021) in a study involving 13,292 serum samples, demonstrated that dogs over 25 kg exhibited a lower *L. infantum* seroprevalence, with autochthonous breeds acting as a protective factor (Rombola et al., 2021). Similar findings were reported in other Mediterranean breeds, such as the Ibizan Hound (Burnham et al., 2020) and Cirneco dell'Etna (Amato et al., 2024), which exhibited protective immunological profile likely (Martínez-Sáez et al., 2024; Marín-García and Llobat, 2022; Macia et al., 2023) linked to genetic factors (Álvarez et al., 2022).

Similarly, *T. gondii* seroprevalence (5.6 %) was lower than reported in previous studies, such as one conducted in Italy that observed a 29.2 % of prevalence (Dini et al., 2024). That study identified cohabitation with cats as a risk factor for *T. gondii* infection due to dog's coprophagic behavior. However, since Fonni's dogs have traditionally been used for livestock protection (Cocco et al., 2024b), they rarely cohabited with cats, reducing their risk of exposure.

Regarding age-related effects, total protein and lipase levels increased with age, while albumin were higher in young and adult dogs. Calcium and phosphorus were highest in puppies. Cholesterol levels decreased after 9 months of age but rose again in senior dogs. In contrast, creatinine levels were lower in puppies. These results aligned with a study on 3045 dogs of varying sex, age, reproductive status, and breed (Chang et al., 2016), which also reported increased ALP activity after 4 years of age. However, in this study, ALP levels were consistently high in puppies and remained stable after 9 months. Highly elevated ALP activity has been associated with liver inflammation due to aging (Steele et al., 2014), although breed-specific differences exist. For instance, Dogues de Bordeaux were found to exhibit decreased ALT activity with age (Lequarré et al., 2011).

The study also evaluated age-related variations in CPK, GOT, and TRI levels. CPK activity was highest in puppies, GOT decreased with age, and TRI increased. Regarding sex differences, males had higher GPT activity than females, a trend also observed in beagles (Ikeuchi et al., 1991). Increased CPK activity was linked to high physical activity, particularly in puppies, while age-related increases in TRI were associated with reduced lipoprotein lipase activity. Notably, high CPK activity was not unique to dogs and had also been observed in humans (Spitler and Davies, 2020).

Reductions in CPK had previously been associated with various infections, including *Rickettsia* spp., *L. infantum* and *E. canis* (Cocco et al., 2024b). However, the findings of this study indicated that *Rickettsia* spp. and *L. infantum* reduced CPK levels, while *E. canis* had no significant effect, and *T. gondii* increased them. Elevated CPK activity is was typically associated with muscle damage and inflammation, especially in early-stage infections (Islam et al., 2021). Conversely, low CPK levels

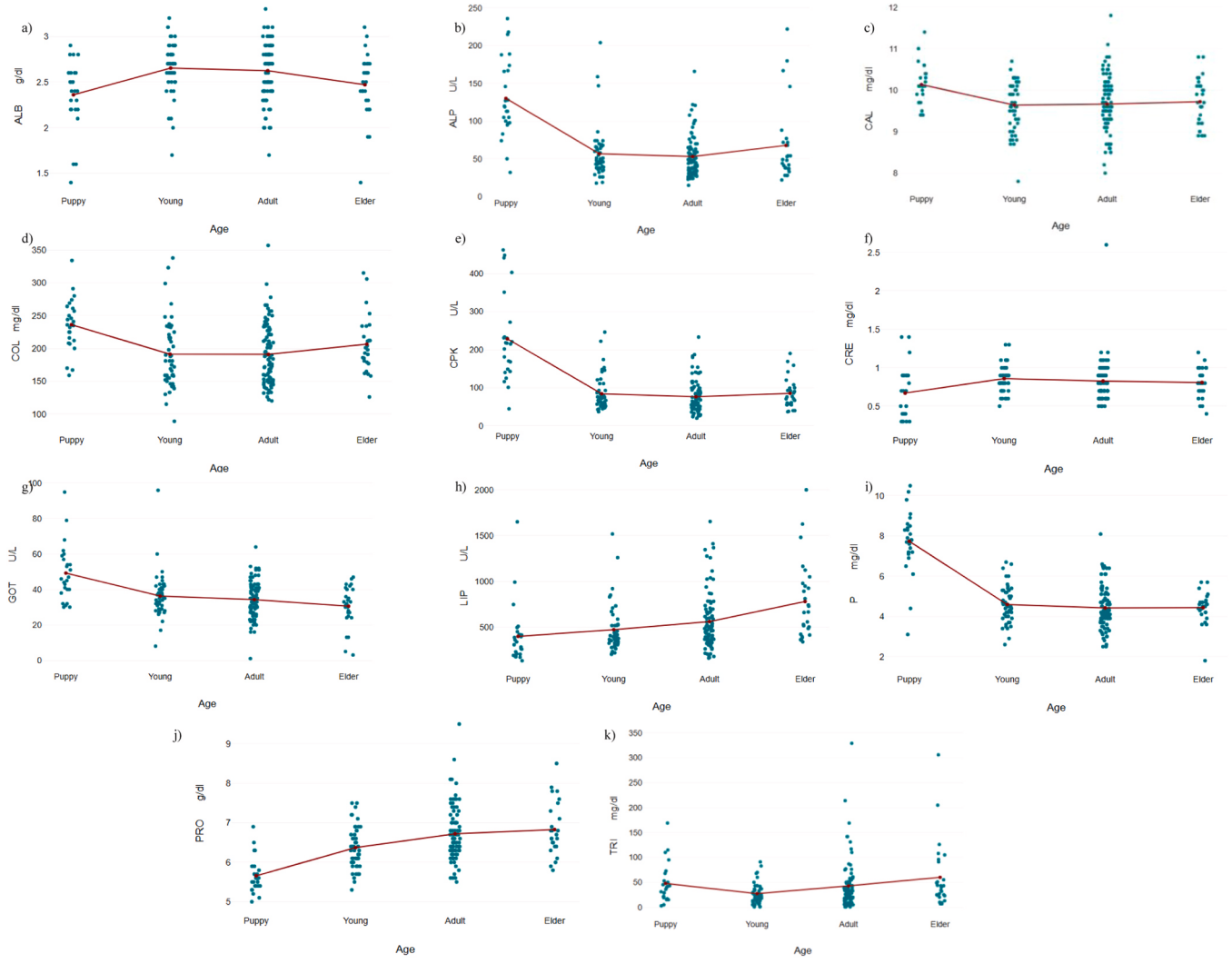

Fig. 1. Biochemical parameters with statistical differences (p 0.05) between puppies, young, adults, and senior dogs included in the study. a) Albumin (ALB), b) Alkaline phosphatase (ALP), c) Calcium (CAL), d) Total cholesterol (COL), e) Creatine phosphokinase (CPK), f) Creatinine (CRE), g) Aspartate aminotransferase (GOT), h) Lipoprotein lipase (LIP), i) Phosphorus (P), j) Total protein (PRO), k) Triglycerides (TRI).

Table 3
Biochemical parameters of infected and coinfected dogs. Data show as mean ± SD. Albumin (ALB), alkaline phosphatase (ALP), amylase (AM), total Bilirubin (BT), calcium (CAL), total cholesterol (COL), creatine phosphokinase (CPK), creatinine (CRE), gamma glutamyl transferase (GGT), glucose (GLU), aspartate aminotransferase (GOT), glutamate-pyruvate transaminase (GPT), lipoprotein lipase (LIP), phosphorus (P), total protein (PRO), triglycerides (TRI), and urea (UR).

Parameters (units)	Number of infections (number of dogs)						
	Non infected (n 47)	**One (n 72)**	**Two (n 53)**	**Three (n 16)**	**Four (n 1)**	**Five (n 1)**	***p*-value**
ALB (g/dL)	2.61 ± 0.32	2.51 ± 0.37	2.63 ± 0.27	2.61 ± 0.30	2.20 ± 0.00	2.90. ± 0.00	0.1539
ALP (unit/L)	81.79 ± 53.44	64.97 ± 38.27	60.53 ± 44.74	50.53 ± 23.57	31.00 ± 0.00	25.00 ± 0.00	0.7244
AM (units/L)	965.81 ± 810.19	859.04 ± 324.41	816.85 ± 232.83	888.19 ± 281.65	737.00 ± 0.00	427.00 ± 0.00	0.6980
BT (mg/dL)	0.06 ± 0.06	0.06 ± 0.05	0.07 ± 0.07	0.06 ± 0.07	0.06 ± 0.00	0.01 ± 0.00	0.8972
CAL (mg/dL)	9.88 ± 0.62	9.62 ± 0.66	9.79 ± 0.61	9.63 ± 0.55	8.70 ± 0.00	10.00 ± 0.00	0.2033
COL (mg/dL)	211.94 ± 50.24	198.93 ± 50.58	189.57 ± 44.49	197.56 ± 38.07	133.00 ± 0.00	221.00 ± 0.00	0.4538
CPK (units/L)	122.04 ± 89.16	100.34 ± 74.55	89.00 ± 63.74	66.19 ± 29.44	58.00 ± 0.00	20.00 ± 0.00	0.7123
CRE (mg/dL)	0.79 ± 0.23	0.83 ± 0.31	0.80 ± 0.19	0.80 ± 0.13	0.90 ± 0.00	0.70 ± 0.00	0.8906
GGT (units/L)	10.43 ± 2.93	10.36 ± 3.19	9.58 ± 3.12	10.00 ± 2.92	15.00 ± 0.00	10.00 ± 0.00	0.3829
GLU (mg/dL)	70.81 ± 11.95	69.01 ± 15.28	72.22 ± 15.70	65.69 ± 15.46	65.00 ± 0.00	66.00 ± 0.00	0.6833
GOT (units/L)	39.11 ± 11.31	37.28 ± 12.82	34.24 ± 9.96	31.94 ± 19.93	16.00 ± 0.00	21.00 ± 0.00	0.2990
GPT (units/L)	60.28 ± 27.71	60.94 ± 34.40	51.60 ± 19.71	61.25 ± 27.33	40.00 ± 0.00	57.00 ± 0.00	0.4964
LIP (units/L)	577.87 ± 356.48	541.36 ± 335.87	523.52 ± 302.90	531.50 ± 269.41	1411.00 ± 0.00	393.00 ± 0.00	0.0742
P (mg/dL)	5.43 ± 1.75	4.84 ± 1.52	4.65 ± 1.42	4.53 ± 0.65	4.20 ± 0.00	3.90 ± 0.00	0.9949
PRO (g/L)	6.35 ± 0.76	6.51 ± 0.79	6.58 ± 0.62	6.72 ± 0.51	6.00 ± 0.00	6.10 ± 0.00	0.8057
TRI (mg/dL)	40.38 ± 50.43	49.47 ± 52.86	35.71 ± 35.19	33.19 ± 28.41	34.00 ± 0.00	41.00 ± 0.00	0.7690
UR (mg/dL)	32.21 ± 16.84	34.46 ± 25.08	27.91 ± 11.96	27.94 ± 15.05	28.00 ± 0.00	19.00 ± 0.00	0.5917

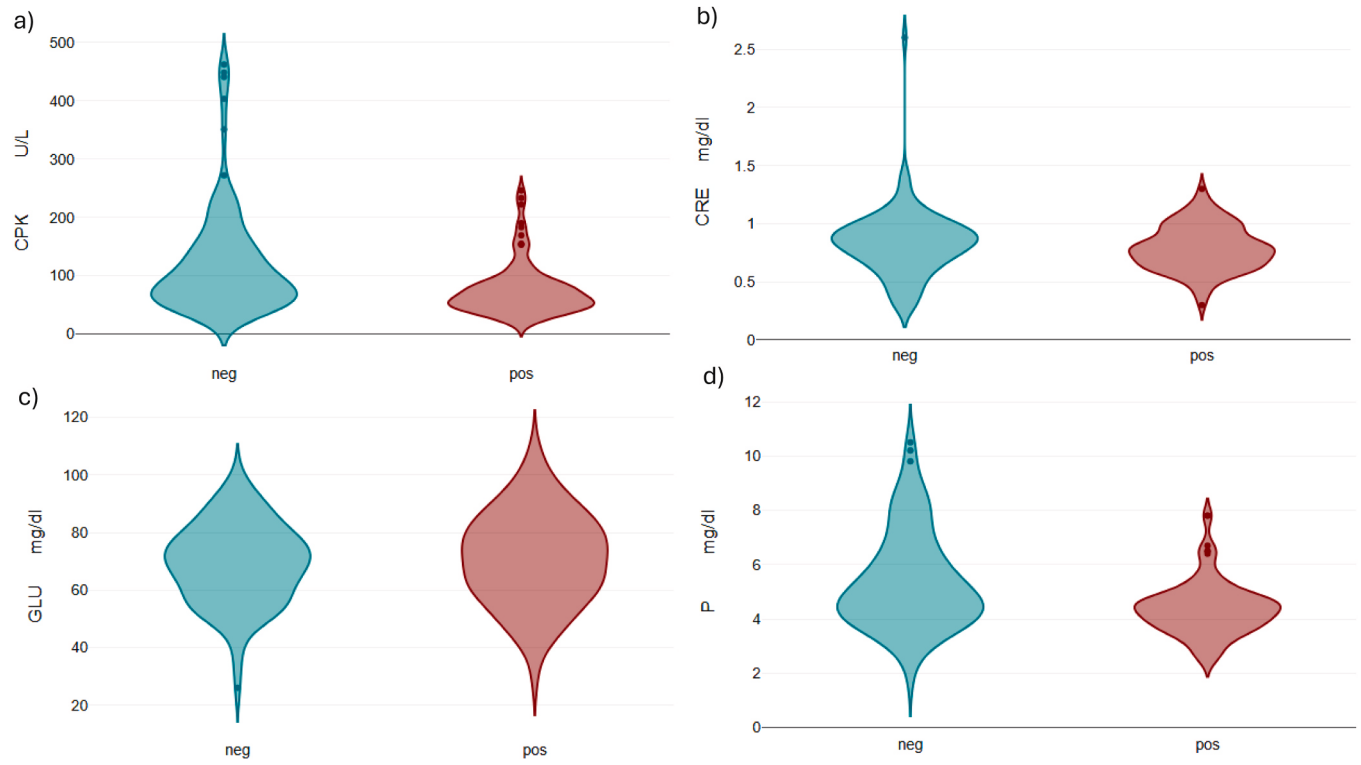

Fig. 2. Biochemical parameters which statistically differ (p-value 0.05) in infected and non-infected dogs for *Rickettsia* spp. a) Creatine phosphokinase (CPK), b) Creatinine (CRE), c) Glucose (GLU), d) Phosphorus (P).

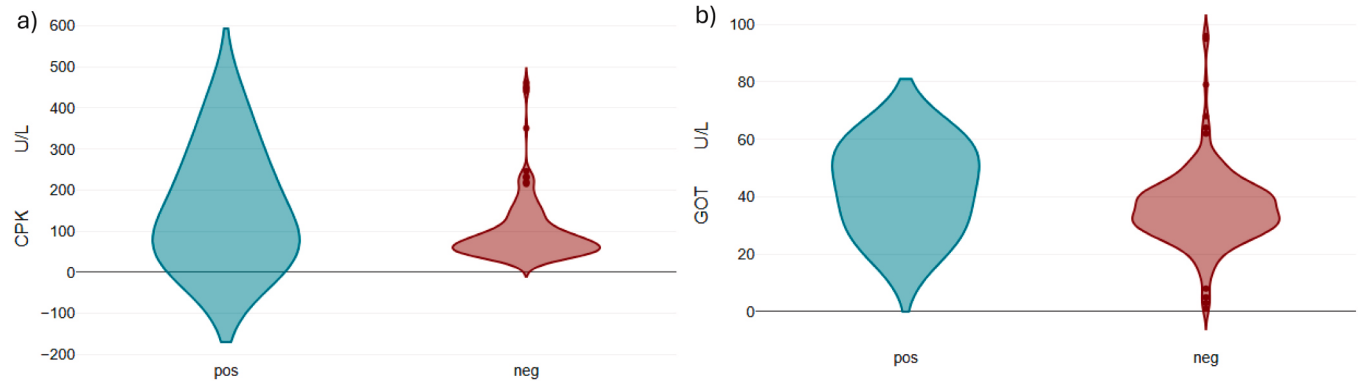

Fig. 3. Biochemical parameters which statistically differ (p-value 0.05) in infected and non-infected dogs for *L. infantum*. a) Creatine phosphokinase (CPK), b) Aspartate aminotransferase (GOT).

had been linked to muscle loss in human diseases (Zygmunt et al., 2023). The reduced inflammatory response observed in Fonni's dogs may suggest a more efficient immune reaction to certain infections. Additionally, *Rickettsia* spp. infection decreased CRE levels, indicating potential muscle mass loss (Sakkas et al., 2009). However, while *L. infantum* infection lowered CPK levels, it did not affect CRE, suggesting that Fonni's dogs may possess an enhanced immune response to *L. infantum*, like other Mediterranean breeds [257].

For the first time, Fonni's dogs infected with different pathogens were evaluated for their haematological profiles. The results showed differences in several parameters compared to those observed in other canine breeds. However, a key limitation of this study was its reliance solely on serological tests, which may result in cross-reactivity antibodies and false positives. Future studies should consider longitudinal approaches to monitor potential clinical signs over time. Additionally, comparative studies involving other breeds would be valuable, along with investigations into the genetic and immunological factors contributing to the observed responses in the Fonni's dog's.

5. Conclusions

Fonni's dogs showed a prevalence of *Rickettsia* spp., *E. canis* and *A. phagocytophylum* similar to that observed in other Mediterranean regions, while *L. infantum* infection was lower than in other canine breeds. Epidemiological factors influenced the haematological profile in both infected and non-infected animals, with age being the most significant factor, whereas the number of coinfections had no notable effect. Haematological parameters were asssociated to infection. For instance, *Rickettsia* spp. infection appeared to result in muscle mass loss, while *L. infantum* infection did not significant alter parameters typically associated with parasitic infections. These findings suggested that the Fonni's dog breed may respond differently to certain infections

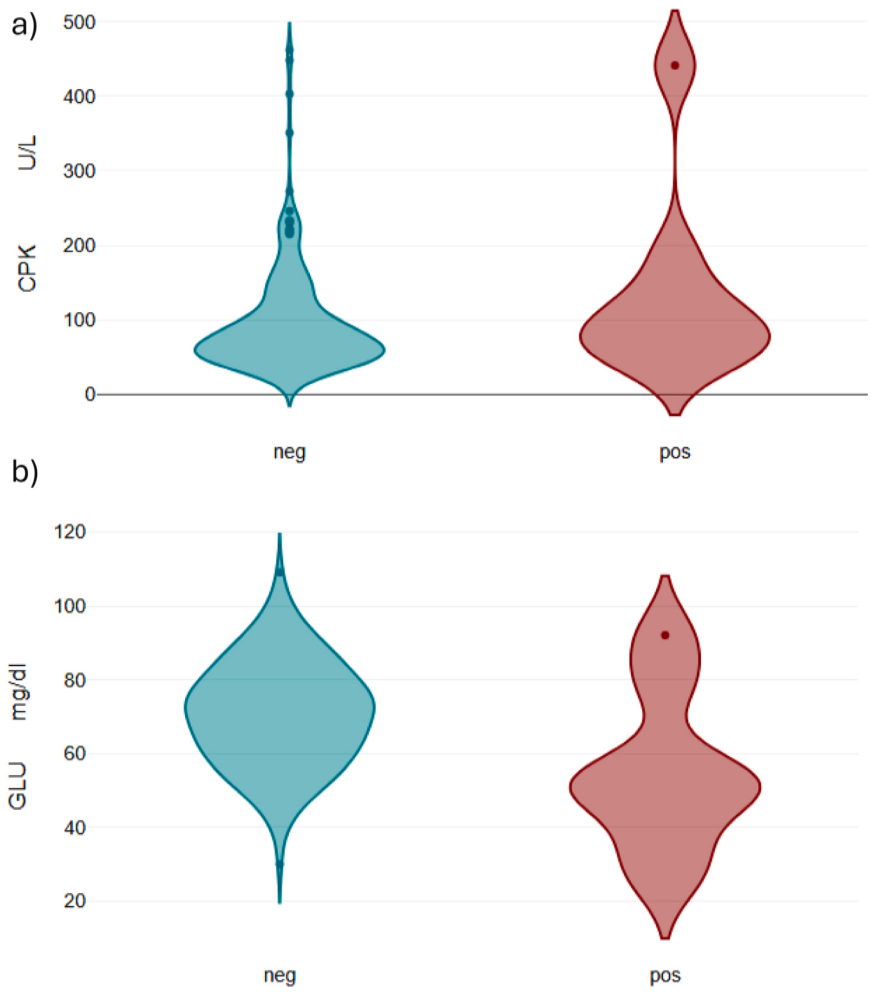

Fig. 4. Biochemical parameters which statistically differ (p-value 0.05) in infected and non-infected dogs for *T. gondii.* a) Creatine phosphokinase (CPK), b) Glucose (GLU).

compared to other dog breeds. In addition, the data indicated that outdoor working dogs were generally seropositive for a variety of canine vector-borne diseases (CVBDs), despite exhibiting no clinical signs. This could be attributed to the limited specificity of serological testing for diagnosing infections, owing to cross-reactivity between pathogens and non-pathogens, the low pathogenicity of some agents in dogs, and the persistence of circulating antibodies long after the initial infection.

Future studies on the immune response to *L. infantum* and other endemic pathogens of the Mediterranean basin are needed in this breed and in other outdoors dogs to better understand the underlying causes of these observed differences.

CRediT authorship contribution statement

Llobat Lola: Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Marín-García Pablo Jesús:** Methodology, Formal analysis. **Liotta Luigi:** Writing – review & editing, Project administration, Data curation, Conceptualization. **Cocco Raffaela:** Methodology, Data curation. **Sechi Sara:** Methodology, Data curation.

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Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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