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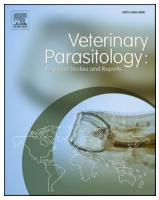

Original Article

Immunological profile of two canine breeds in an endemic region of *Leishmania infantum*

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ABSTRACT

Visceral leishmaniosis is the one of the most important protozoal zoonoses in Europe, and it is caused by *Leishmania infantum*, an intracellular protozoan parasite. The disease is endemic in dogs in the Mediterranean area. The main goal of this work is to correlate the levels of several cytokines linked to immune response against L. *infantum* infection in two canine breeds. Thirty-one Boxer and twenty-eight Ibizan Hound dogs living in the Valencian Community (East coast of Spain) were analyzed for the presence of anti-*Leishmania* antibodies in serum by IFAT test. Cytokines IFN-γ, TNF-α, IL-2, IL-6, IL-8, and IL-18 were determined by ELISA commercial tests. The levels of IFN-γ, IL-2, and IL-18 in our study, cytokines linked to a cellular immune response, were higher ($p < 0.05$) in the Ibizan Hound breed; IL-6 levels were higher, although not significant, and only levels of IL-8 were higher in Boxer than in Ibizan Hound. No expression of TNF-α was found. These results corroborate that Ibizan Hound can develop a protective response against canine leishmaniosis, while Boxer is a susceptible breed. The study of immunological aspects in the different canine breeds may represent a useful tool in the prediction of the disease.

1. Introduction

Leishmaniosis is a zoonotic disease caused by the infection with the protozoan parasites *Leishmania* spp. (order Kinetoplastida, family Trypanosomatidae), which is transmitted by the bite of phlebotomine sand flies from the Psychodidae family, being the *Phlebotomus* spp. their vectors in the Mediterranean region (Baneth et al., 2008; Gramiccia, 2011; Akhoundi et al., 2016). In humans, this parasitosis can be presented in its mucocutaneous or visceral form, being the latter the most severe and caused in the same geographical region by the species *Leishmania infantum* (Barbiéri, 2006; Ready, 2014; Trájer and Sebestyén, 2019). The domestic dog (*Canis lupus familiaris*) is the main reservoir of this protozoan, although it has been isolated from many other species (Mancianti et al., 1988; Ready, 2014).

The immune response generated after infection by L. *infantum* can vary between hosts and determines the severity of the disease (Vanloubbeeck and Jones, 2004; Solano-Gallego et al., 2009; Rossi and Fasel, 2018). Once the parasite has been inoculated by the sandfly bite, the macrophages of the host lead the L. *infantum* antigen to undifferentiated Th0 lymphocytes, which can induce two types of response (Barbiéri, 2006; Hosein et al., 2017). One of them is the activation of Th1 lymphocytes which triggers a cellular response with the synthesis of free oxygen radicals, and destruction of the parasite by macrophages. The cytokines involved in this pathway are interleukin 2 (IL-2), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) (Barbiéri, 2006; Solano-Gallego et al., 2009; Hosein et al., 2017). This response makes it possible to control the infection, and the host does not show clinical signs of the disease (Pinelli et al., 1994). Ordeix et al. (2018, 2019, 2020) showed that infected animals with low parasite loads and clinically healthy had higher levels of IFN-γ compared to sick dogs. The other response is the pathway mediated by Th2-type lymphocytes, which triggers the humoral immunity, with the stimulation of interleukins 4, 5, and 10 (IL-4, IL-5, and IL-10), producing an excess of antibodies (Pinelli et al., 1994; Barbiéri, 2006; Hosein et al., 2017). This humoral response is ineffective in killing the parasites, since they are inside the macrophages, so the disease progresses and clinical signs finally appear (Pinelli et al., 1994; Ordeix et al., 2018;). Furthermore, the IL-10 inhibits the Th1 response, which inactivates the macrophages

Abbreviations: IFAT, immunofluorescent antibody; IFN, Interferon; IL, Interleukin; ELISA, Enzyme-Linked ImmunoSorbent Assay; TNF, Tumor necrosis factor; HRP, Avidin-Horseradish peroxidase; OD, Optimal density; Th, T helper; LSA, *L. infantum* Soluble Antigen.

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and makes those apparently healthy hosts show a lower production of lymphocytes, as well as an absence of IFN-γ (Barbiéri, 2006). Other biomarkers are involved in the immune response against canine leishmaniosis. Several studies showed that the infection by L. *infantum* causes a signaling cascade that leads to the production of different chemokines, including CXCL1, responsible for recruiting neutrophils (Bozic et al., 1995; Solcà et al., 2016). Dogs naturally and experimentally infected exhibited an increase in cytokines IL-6, IL-18, and IFN-γ levels, and a decrease in cytokines TNF-α, IL-2, and IL-8 levels (Abbehusen et al., 2017).

According to several studies, the activation of one or another immune response could be different depending on the canine breed (Solano-Gallego et al., 2000a; Sanchez-Robert et al., 2005; Martínez-Orellana et al., 2017a; de Vasconcelos et al., 2019; Edo et al., 2021). Specifically, the Ibizan Hound has a higher concentration of IL-6 and IFN-γ than other pure breed dogs or crossbreeds (Martínez-Orellana et al., 2017b). The IL-6 is a cytokine with a rapid and transient activation after infections and it contributes to the host protection through the stimulation of acute response (Tanaka et al., 2014). An increase in these interleukin levels has been observed in the early stages of the infection with L. *infantum* (Abbehusen et al., 2017). The IFN-γ is a type I interferon, which acts as a mediator in the innate immune response with protective activity against different viruses (Cook et al., 2019). Also, IFN-γ levels are higher in infected L. *infantum* dogs compared to uninfected, so has been linked to the control of the proliferation and the spread of the parasite in the host (Abbehusen et al., 2017). Some studies point to serum levels of IFN-γ as a possible biomarker of the infection before clinical signs appear (Zribi et al., 2017). The specific cellular response of *Leishmania* spp. the infection showed to be higher in the Ibizan Hound breed than in other canine breeds. In fact, (Solano-Gallego et al., 2000a) demonstrated that Ibizan Hound dogs have a higher cellular immune response than dogs of other breeds, which makes them more resistant to clinical leishmaniosis. Sanchez-Robert et al. (2008a) conducted a study with nineteen canine breeds, including Ibizan Hound and Boxer, where they analyzed the presence of polymorphisms in the Slc11a gene, linked to susceptibility to canine visceral leishmaniosis. The genetic origin could be related to both innate and adaptative immune responses and determine different immune pathways (Altet et al., 2002; Sanchez-Robert et al., 2008b). Recently, Edo et al. (2021) showed a higher prevalence of clinical leishmaniosis in Boxer and Doberman Pinscher than in other canine breeds.

The aim of this study is to evaluate the levels of several cytokines linked to cellular and humoral response against leishmaniosis in two canine breeds, the Ibizan Hound and Boxer living in an endemic region of L. *infantum*.

2. Materials and methods

2.1. Ethics approval

The experiments involving animals were conducted according to the guidelines of the Declaration of Helsinki and approved by the Animal Experimentation Ethics Committee of the CEU Cardenal Herrera Universities, with code 2020/VSC/PEA/0216. Dog owners were informed of the risks and aim of the study and signed a written informed consent for sampling them.

2.2. Animals and data collection

From October 2021 to May 2022, fifty-nine dogs living in the Valencian Community (thirty-one Boxers and twenty-eight Ibizan Hounds) were sampled for the study. All the animals were pure breed and belonged to Ibizan Hound Association and Boxer Club of Valencia. For every animal, the following epidemiological data were registered: 1) sex, 2) age (four categories: puppies -less than one-year-old-, young -between one and five years old-, adult -between five and ten years old-, and elder -more than ten years old), 3) official vaccination in order (two categories: yes or not), 4) leishmaniosis vaccination (two categories: yes or not), 5) use of ectoparasiticides (two categories: yes or not), 6) living with other animals (two categories: yes or no; if yes, the species of animals lives were annotated), 7) type of food (two categories: raw food or commercial food), 8) type of life (two categories: indoor or outdoor dogs), 9) antibody titre for L. *infantum* (four categories: negative (titre <1/80), positive with low or questionable titre (<1/100), medium (medium (between 1/100 and 1/400), and elevated (>1/400). All animals included in the survey were clinically healthy.

2.3. Samples collection and cytokines analysis

Ten milliliters of whole blood were taken by cephalic venipuncture with Vacutainer tubes without anticoagulant. Samples were maintained at room temperature to obtain serum aliquots, which were stored at −20 °C until processing. Serological testing for L. *infantum* detection of specific antibodies was performed using the indirect immunofluorescent antibody test (IFAT) for anti-*Leishmania*-specific immunoglobulin G (IgG) antibodies (MegaFLUO® LEISH, Megacor Diagnostik GmbH, Hörbranz, Austria). Samples were considered seropositive with IFAT titre ≥1/80, following the manufacturer's instructions (Olías-Molero et al., 2019).

Serum levels of TNF-α, IFN-γ, IL-2, IL-6, IL-8, and IL-18 were measured by a commercial kit of indirect ELISA assay (Canine TNF-α ELISA kit, Canine IFN-γ, Canine IL-2 ELISA kit, Canine IL-6 ELISA kit, and Canine IL-8 ELISA kit, Invitrogen, and Canine IL-18 ELISA kit, MyBioSource, NY, EEUU), following the manufacturer's recommendations. The sensitivity and precision intra- and inter-assay are shown in Table 1. Briefly, a 50–100 μL of serum was added to the microplate wells. Then, a biotinylated detection antibody specific for different cytokines and Avidin-Horseradish peroxidase (HRP) conjugated was added successively to each microplate well and incubated. Free components were washed away, and the substrate solution was added to each well. The enzyme-substrate reaction was determined by the optical density (OD) and measured spectrophotometrically at a wavelength of 450 nm in the plate reader Victor-X3™ (Perkin Elmer®). The concentration of each cytokine was calculated by comparing the OD of the samples to the standard curve.

2.4. Statistical methods

Epidemiological data and serum levels of cytokines were analyzed using the general linear model procedure (PROC GLM) of the statistical package SAS (North Carolina State University, USA) for each cytokine. The normality and the homoscedasticity were tested by Shapiro-Wilks and Levene tests, respectively. The model was carried out with sex,

Table 1
Assay range, sensitivity and precision of ELISA kits used for serum cytokines levels detection.

Cytokine	Assay range	Sensitivity	Precision	
			CV1 Intra-assay	CV1 Inter-assay
TNF-α	2.870–700 pg/mL	2.000 pg/mL	<10%	<12%
IFN-γ	0.102–25 ng/mL	0.100 ng/mL	<10%	<12%
IL-2	0.400–100 ng/mL	0.400 ng/mL	<10%	<12%
IL-6	0.102–25 ng/mL	0.100 ng/mL	<10%	<12%
IL-8	15.625–1000 pg/mL	9.375 pg/mL	<10%	<12%
IL-18	15.600–1000 pg/mL	5.900 pg/mL	<10%	<12%

age, and breed as fixed effects. The statistical significance was set at a p-value<0.05.

3. Results

Of the total analyzed animals, 50.84% were males (30/59) and 49.16% were females (29/59); 10.71% of them (6/59) were puppies (less than one-year-old), 27.12% (16/59) were young (age ranged between one to five years old), 38.98% (23/59) were adults (between five to ten years old), and 23.73% (14/59) were elder (more than ten years old). All animals had obligatory vaccination correctly applied, used ectoparasiticides, and were outdoor dogs. Most individuals (57 to 59) lived with other animals and were fed commercial food (51 to 59). Seventeen dogs (28.81%), all of them Boxer breed, were vaccinated against *Leishmania* (Table 2).

Four Boxers (12.9%) were positive for *Leishmania* antibodies (antibody titre >1/80). Of them, the highest titre (1/320) belonged to an adult male which lived with cats. The other three had a low titre (1/80). Epidemiological data of Boxers with positive test for *Leishmania* antibodies are shown in Table 3. No Ibizan Hound dogs showed a positive result for the IFAT test.

The levels of cytokines for the total population ranged as shown in Table 4. No expression of TNF-α was found. Statistical analysis revealed that breed was the only factor with a significant fixed effect when levels of different cytokines were compared (Table 5). IFN-γ, IL-2, and IL-18 showed higher levels in Ibizan Hound than in Boxer ($p < 0.01$). IL-6 levels were higher, although not significant, also in Ibizan Hound ($p = 0.2611$). Only levels of IL-8 were significantly higher in Boxer than in Ibizan Hound (Fig. 1).

4. Discussion

The results of the present work show the differences in the immunological profiles in Ibizan Hound and Boxer dogs living in an endemic region for L. *infantum*, comparing their levels of different selected cytokines. To the best of the authors' knowledge, IL-2, IL-8, and IL-18 are studied for the first time in these two breeds. Despite the discrepancy derived from previous works, due to the variability in the clinical manifestations, cytokines parameters have deeply been evaluated in dogs, either naturally or experimentally infected with *Leishmania* (Maia and Campino, 2018). As previously suggested, the breed seems to be a factor related to the immune response in clinical leishmaniosis. In the present study, all the Ibizan Hound dogs present IFAT titres not compatible with infection. It has been stated that this breed has a natural genetic resistance against the disease related to the early activation of the cellular immune response, and certain specific cytokines (Solano-Gallego et al., 2000a; Sanchez-Robert et al., 2008b; Martínez-Orellana et al., 2017a; Soutter et al., 2019). None of the Ibizan Hounds analyzed in this survey were seropositive, which contrasts with the work of Sanchez-Robert et al. (2008a) who studied Ibizan Hounds from the Balearic Islands (Spain) and quantified a prevalence of 48.4% seropositive dogs of this breed. However, four Boxers analyzed by us showed antibodies against the parasite. Likewise, this breed with second highest prevalence (after the Doberman pinscher) in a recent study conducted in Ibiza, Balearic Islands (Edo et al., 2021). It is known that Boxer is a breed with a predisposition to *Leishmania* infection (Sanchez-Robert et al., 2005, 2008b). In fact, the breed was the only factor that revealed significant differences when the six cytokines quantified in the present study were compared.

We found that levels of IFN-γ, IL-2, and IL-18 were significantly higher in the Ibizan Hound breed. Whereas IL-6 levels were higher, although not significant, and only levels of IL-8 were significantly higher in Boxer than in Ibizan Hound. No expression of TNF-α was observed. The ability of the host to control *Leishmania* infection requires a strong cellular immune response, associated with the activation of T helper (Th)-1 cells producing IFN-γ, IL-2, and TNF-α (Maia and Campino, 2012, 2018). Some studies about the first two noticed their relationship with a control and a protective response against *Leishmania* infection by the host (Carrillo and Moreno, 2009; Ordeix et al., 2019, 2020), although controversial results have been found in the literature. The IFN-γ production in infected dogs was correlated with resistance to disease, asymptomatic status, or mild disease, with higher levels in infected L. *infantum* dogs compared to uninfected ones (Manna et al., 2008; Solano-Gallego et al., 2016; Abbehusen et al., 2017). Besides, sick, and symptomatic animals showed an absence of this cytokine in peripheral blood (Carrillo and Moreno, 2009; Solano-Gallego et al., 2016). IFN-γ has been proposed as an important prognostic tool for immune monitoring in canine leishmaniosis (Martínez-Orellana et al., 2017a), although some authors did not find it a good marker of resistance against the disease (Travi et al., 2009). Ibizan hound present high levels of IL-2, and IL-18, two cytokines related to cellular immune response. The results found in our study are in accordance with the hypothesis that the Ibizan hound dogs activates the cellular immune response, which causes an invagination of the parasite by the macrophages and prevents its dissemination in the organism (Barbiéri, 2006). Levels of IL-2 seem to be lower in dogs showing mild disease (Pinelli et al., 1994; Hosein et al., 2015; Ordeix

Table 2
Epidemiological data recovered of the animals studied.

Variable	Categories	N° of Ibizan Hound	N° of Boxer	No. of total dogs (%)
Gender	Male	16	14	30 (50.84)
	Female	12	17	29 (49.16)
Age	Puppy (<1 year)	2	4	6 (10.17)
	Young (1 to 5 years)	6	10	16 (27.12)
	Adult (5 to 10 years)	9	14	23 (38.98)
	Elder (>10 years)	11	3	14 (23.73)
Diet	Commercial	23	28	51 (86.44)
	Home prepared/raw food consumption	5	3	8 (13.56)
Lived with other animals	Yes	28	29	57 (96.61)
	No	0	2	2 (3.39)
Anti-*Leishmania* vaccination	Yes	0	27	27 (45.76)
	No	28	4	32 (54.24)
Overall				56 (100.00)

Table 3
Epidemiological data recovered of the animals with positive titre for *Leishmania* antibodies (≥1/80).

Case ID	Sex	Age	Leishmaniosis vaccination	Lived with other animals	IgG titre
1	Male	Adult (5 to 10 years)	No	Cat	1/320
2	Female	Young (1 to 5 years)	No	Dog	1/80
3	Male	Elder (>10 years)	No	Dog	1/80
4	Female	Adult (5 to 10 years)	No	Dog	1/80

Table 4
Range, mean ± standard deviation (SD), and coefficient of variation (CV) of the cytokines analyzed in the total dogs studied.

Cytokine	n	Range	Mean ± SD	CV (%)
IFN-γ (ng(mL)	53	0.01–1.29	0.34 ± 0.04	88
IL-2 (ng/mL)	56	10.37–117.00	55.62 ± 3.40	46
IL-6 (ng/mL)	53	0.40–0.79	0.58 ± 0.09	16
IL-8 (pg/mL)	53	0–577.00	184.00 ± 17.58	70
IL-18 (pg/mL)	53	0–863.00	260.00 ± 38.29	107

Table 5
Mean ± standard deviation (SD) and *p*-value obtained in statistical analysis between epidemiological factors (sex and age) and cytokine serum levels.

		Sex			Age				
		Male	Female	*p*-value	Puppy (<1 year)	Young (1 to 5 years)	Adult (5 to 10 years)	Elder (>10 years)	*p*-value
Cytokine serum levels (mean ± SD)	IFN-γ (ng/mL)	0.38 ± 0.10	0.48 ± 0.10	0.5081	0.26 ± 0.23	0.33 ± 0.14	0.43 ± 0.13	0.56 ± 0.13	0.5488
	IL-2 (ng/mL)	72.84 ± 13.48	63.83 ± 13.14	0.6335	54.01 ± 29.51	59.90 ± 18.21	85.80 ± 17.40	63.76 ± 16.37	0.6754
	IL-6 (ng/mL)	0.85 ± 0.13	0.57 ± 0.13	0.1303	0.52 ± 0.30	0.64 ± 0.18	0.67 ± 0.18	0.85 ± 0.17	0.7217
	IL-8 (pg/mL)	220.00 ± 24.45	225.00 ± 23.84	0.8834	135.00 ± 52.54	205.00 ± 32.43	235.00 ± 30.99	252.00 ± 29.14	0.2470
	IL-18 (pg/mL)	267.00 ± 64.90	299.00 ± 59.20	0.7175	184.00 ± 133.10	263.00 ± 81.50	275.00 ± 72.90	372.00 ± 90.40	0.5913

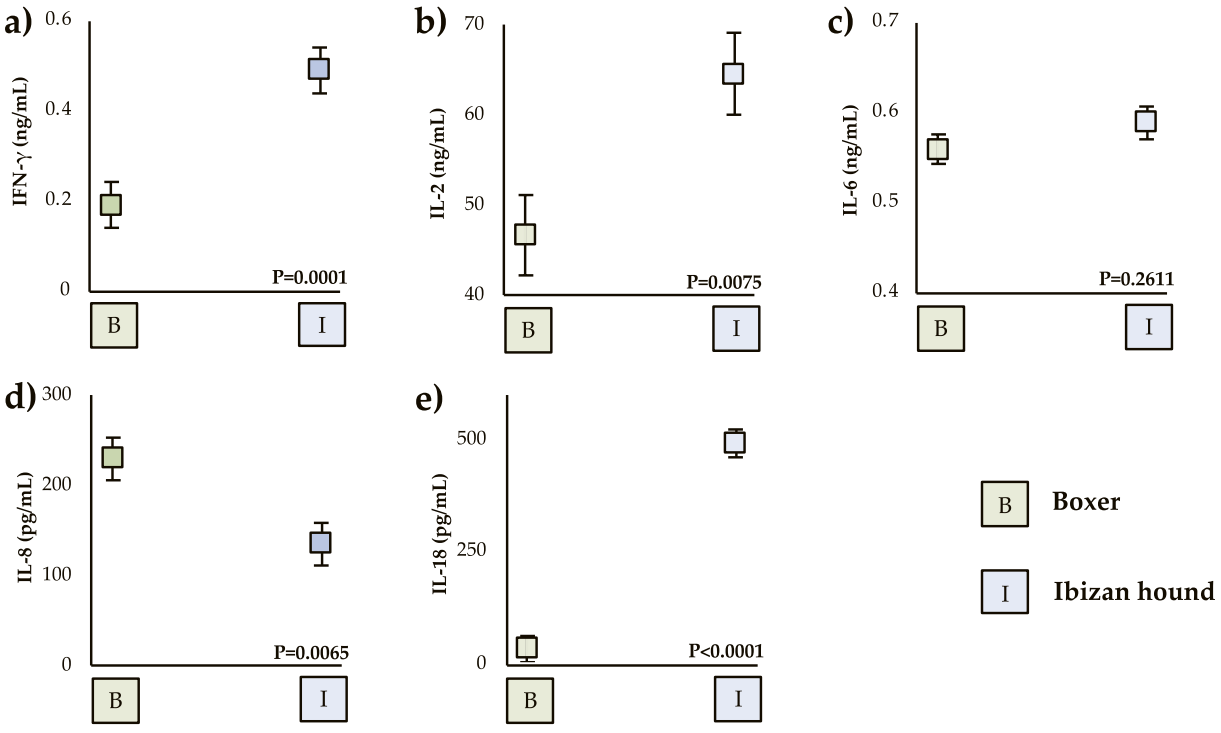

Fig. 1. Serum levels of cytokines in Boxer (B) and Ibizan Hound (I). a) Interferon gamma (IFN-γ), b) Interleukin 2 (IL-2), c) Interleukin 6 (IL-6), d) Interleukin 8 (IL-8), and e) Interleukin 18 (IL-18). Squares represent values for two breeds, and vertical lines represent standard deviation. Values for IL-8 and IL-8 are expressed in pg/mL, and for IFN-γ, IL-2, and IL-6 in ng/mL. Different *p*-values for cytokines are shown in figures a, b, c, d, and e.

et al., 2018). Aslan et al. (2016) found that IL-2 expression was negatively correlated with splenic parasite loads in experimentally infected dogs. Our results are by the proposal made by most of the authors, which suggest a natural resistance in Ibizan Hound against CanL when compared with more susceptible dog breed, like Boxer (Solano-Gallego et al., 2000a; Sanchez-Robert et al., 2008b; Martínez-Orellana et al., 2017b, 2022). IL-18 is a cytokine that plays an important role in both types of immunity, innate and acquired, and mediates Th1 response by inducing IFN-γ production in T cells and natural killer cells (Okamura et al., 1998; Takeda et al., 1998). Kumar et al. (2014) found a significant relationship between susceptibility to visceral leishmaniosis in human and high levels of this cytokine. However, some authors pointed out that it seems to have no role in the infection outcome of the disease in dogs (Pinelli et al., 1994; Chamizo et al., 2005; Manna et al., 2006; Carrillo et al., 2007; Aslan et al., 2016). Levels of the inflammatory cytokine IL-6 were higher (although not significant) in the Ibizan Hound than in Boxer dogs. In accordance, Chamizo et al. (2005) and Martínez-Orellana et al. (2017b) found a stronger expression of IL-6 in healthy dogs when compared with experimentally infected sick dogs. Their levels increased in animals after natural and experimental infection (Abbehusen et al., 2017). de Lima et al. (2007) pointed out this cytokine as a good marker in the active disease. Other authors did not find relation between these cytokine levels and the development of leishmaniosis (Pinelli et al., 1994; Aslan et al., 2016). On the contrary, IL-8 was significatively higher in the Boxer breed in the present work. These results are consistent with the scarce studies focused on the role of this interleukin in *Leishmania* infection, so is an important cytokine for the activation of immune cells, especially neutrophils. Wardini et al. (2019) found higher basal levels of IL-8 production in neutrophils from infected dogs when compared with healthy animals. In humans, IL-8 polymorphism has been considered a risk factor for the development of visceral leishmaniosis (Hajilooi et al., 2015). A decrease in its levels has been noted in dogs infected, both naturally and experimentally, with the protozoan (Abbehusen et al., 2017). Besides, it has been stated that amastigotes accumulate and survive in neutrophils in animals with high IL-8 levels, although the studies developed are not conclusive (Sanz et al., 2021). Finally, no

expression of TNF-α was detected by the ELISA technique in any of the animals analyzed. Opposite to it, the lymphocytes production of this cytokine were significantly lower in symptomatic animals than lymphocytes of uninfected and asymptomatic dogs (Pinelli et al., 1994). However, a Th1 or Th2 immune response (with under- or over-production of TNF-α and IFN-γ, respectively) have been correlated with absence or presence of clinical signs and an increased parasite load in different organs and tissues and clinical disease (Maia and Campino, 2018). Other authors working with Ibizan Hound dogs found significantly higher levels of TNF-α after stimulation with L. *infantum* Soluble Antigen (LSA) in this breed than in controls (Martínez-Orellana et al., 2017b). However, the levels of TNF-α in Ibizan Hound group without stimulation were around 0 to 20 pg/mL (Martínez-Orellana et al., 2017b). So, the sensitivity of ELISA kit used in our study was 2 pg/mL (see Table 1), being this a possible explanation of no expression of this cytokine found in our samples. The undetectable values of TNF-α and the high values of IFN-γ in Ibizan Hound serum samples could be explained by the activation of a different immune response in Ibizan Hound dogs when they are exposed to the parasite. Ibizan Hounds present a predominant T-cell-mediated immune response with the production of Th1 cytokines (including IFN-γ, IL-2, and IL-18). These results could be support the hypothesis that the autochthonous purebred dogs in endemic regions such the Mediterranean, have evolved developing a resistance to the disease (Solano-Gallego et al., 2000b). Similarly, the Sardinian sheepdog and the Maremma sheepdog showed low levels of seroprevalence in the same region (Dreger et al., 2016; Rombolà et al., 2021). Several epidemiologic surveys noticed higher prevalence in Boxers, German shepherds, Doberman pinschers, cocker Spaniels, and among American and English foxhounds (Gaskin et al., 2002; França-Silva et al., 2003; Sanchez-Robert et al., 2005; Edo et al., 2021). Other surveys demonstrated the relationship between CanL and crossbreed, which was pointed out as a protective factor (Cortes et al., 2012; Maia et al., 2017). Regarding other epidemiological factors analyzed, we found no relationship between age or sex and levels of cytokines. Interestingly, the dog with the higher title in our survey was an elder male Boxer breed. Previous studies stated that older dogs developed a more severe disease, blood parasitemia, higher antibody levels, and lower IFN-γ than healthy dogs (Montserrat-Sangrà et al., 2018; Rombolà et al., 2021). In addition, several studies indicated that the males showed higher seroprevalence rates than females in accordance with this data (Rombolà et al., 2021; Tamponi et al., 2021).

5. Conclusions

Susceptibility to *Leishmania* infection varies among individuals, being related to the genetic and immune profiles. In this sense, most of the studies were based on human and murine models, although there are scarce works focused on canine leishmaniosis. In dogs, the breed has been pointed as an important factor in the development of the disease. While some animals can eliminate the parasite or control the parasite burden, remaining subclinical, others show many clinical signs and a very poor prognosis. Our results show higher serum levels of cytokine related to cellular immune response in Ibizan hound than Boxer. More studies are needed in this unexplored field, including other autochthonous and purebred dogs. However, the overall results demonstrated that the evaluation of seroprevalence and immunological mechanisms can represent a reliable tool for predicting the disease. Thus, the identification of specific markers that are involved in the control of leishmaniosis may contribute to the development of more effective therapeutic and prophylactic tools and increase the effectiveness of vaccines.

Ethical statement

The animal study protocol was approved by the Animal Experimentation Ethics Committee of the Universidad Cardenal Herrera CEU (protocol code 2020/VSC/PEA/0216) for studies involving animals.

Declaration of Competing Interest

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