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Article

Interleukin 6 and Interferon gamma haplotypes are related to cytokine serum levels in dogs in an endemic *Leishmania infantum* region

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Simple Summary: Leishmaniasis is a zoonotic disease, endemic in 88 countries, including those from the Mediterranean region. Several authors indicate differences in susceptibility and resistance to leishmaniosis in different canine breeds. Ibizan Hound (an autochthonous canine breed of the Mediterranean region) present resistance to *Leishmania* infection whereas Boxer is one of the most susceptible canine breeds. This study analyzes the serum profiles of cytokines related to the immune response, together with the screening of genomic variants fixed in Boxer and Ibizan Hound dogs in an *Leishmania infantum* endemic region, to understand their differential resistance or susceptibility to *L. infantum* infection. The results of this study indicate several haplotypes in genes encoding *IFNG* and *IL6* are correlated with serum levels of IFN-γ, IL-2 and IL-18, traditionally related to resistance against *Leishmania* infection.

Abstract: Background: The Ibizan Hound is a canine breed native to the Mediterranean region, where leishmaniasis is an endemic zoonosis. Several studies indicate a low prevalence of this disease in dogs, whereas other canine breeds present a high prevalence. However, the molecular underlaying mechanisms yet remains unknown. Methods: In this study, we analyze the haplotypes of genes encode cytokines related to immune response of *Leishmania infantum* infection in twenty-four Boxer and twenty-four Ibizan hound apparently healthy using CanineHD DNA Analysis BeadChip including 165,480 mapped positions. Results: The results show that several haplotypes of genes encoding Interleukin 6 (*IL6*) and Interferon gamma (*IFNG*) are related to Interferon gamma (IFN-γ), and Interleukins (IL) 2 and 18 serum levels. Our results indicate that the regulation of immune response is different in the two canine breeds analyzed and are related to the haplotype compositions of the genes encoding these cytokines. Conclusions: Future studies are needed to elucidate whether these differences and haplotypes are related to different phenotypes in immune response and expression gene regulation to *L. infantum* infection in dogs.

Keywords: cytokines; haplotypes; immune response; *Leishmania*; regulatory mechanisms; resistance; susceptibility

1. Introduction

Leishmaniasis is a vector-borne zoonotic disease caused by infection with the obligate intracellular protozoan parasite in the family Trypanosomatidae, order Kinetoplastida, genus *Leishmania* [1], which are transmitted by the phlebotomine sandflies from the Psychodidae family [2]. This disease could present with different clinical manifestations, which are classified into mucocutaneous (ML), cutaneous (CL) or visceral (VL) form (kala-azar disease), being the last one the most pathogenic and caused by *L. donovani* in Asia and Africa, and by *L. infantum* in the Mediterranean Basin, the Middle East, Central Asia,

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South America, and Central America [3]. The VL produces in humans around 20,000 to 40,000 deaths and 200,000 to 400,000 new infections per year, being one of the most relevant parasitic diseases [4]. Although the parasite has been recently found in different species as reptiles [5], wild carnivores [6,7], wild rabbits [8,9], horses [10] and cats [11], the most relevant host of *L. infantum* is the dog, where caused canine leishmaniasis (CanL) [12]. Seroprevalence of *L. infantum* infection is related to different factors, with controversial results. For example, Gálvez et al. (2020) and Rombolà et al. (2021) found higher prevalence in males than females, and in younger dogs than older ones, whereas Varjao et al. (2021) does not found association between seropositivity and sex, but describe a higher prevalence in older dogs than younger ones [13–15]. Different seroprevalence related to canine breed is commonly referred in several papers, being higher the prevalence in Doberman Pinscher or Boxer when compared to the autochthonous canine breeds of endemic areas [14,16]. One of them is the Ibizan hound, an autochthonous canine breed of Balearic Islands, which appears to be resistant to *L. infantum* infection compared to other breeds [17]. In fact, Solano-Gallego et al. (2000) show a significant cellular response to infection in this canine breed [18]. Cellular response mediated that Th1 response is related to several cytokines production, concretely, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin 2 (IL-2), which activate the macrophage that eliminates the parasite [12,19]. Other cytokines such as IL-4, IL-10 and transforming growth factor beta (TFG-β) activates the Th2 response (humoral immune response) and provokes the dissemination of parasite [20]. Abbehusen et al. (2017) related to *L. infantum* infection with CXCL1 production, which produces a cellular immune response and increases levels of several cytokines such as IFN-γ, IL-6 and IL-18, whereas decrease TNF-α, IL-2 and IL-8 levels [21]. Thus, canine breeds resistant to infection could present different levels of other cytokines than IFN-γ, which provokes the activation of Th1 response. Furthermore, the genetic factors associated with cytokines levels and resistance to *L. infantum* infection have not been studied. A few studies have been realized in this point and none of them related to Ibizan hound. Concretely, twenty-four polymorphisms have been analyzed in the Slc11a1 gene in 40 dogs of different canine breeds, and two of them were associated with increased risk for CanL [22]. *Scl11a1* gene is related to autoimmune and infectious diseases in humans, so fibrosis progression in hepatitis C, Crohn's disease, type-1 diabetes mellitus and tuberculosis [23–26]. In *L. infantum* infection, this gene controls the replication of intracellular parasites [27], and its haplotypes TAG-9-145 and TAG-8-141 are more frequently in Boxer dogs than other canine breeds and, given that the CanL is elevated in this breed, the authors conclude that this gene could be related to leishmaniasis susceptibility [28]. Quilez et al. (2012) realized a genome-wide association study in the same canine breed and found a region in chromosome 4 which could present several markers with greatest effect on the susceptible phenotype [29]. However, none of these studies have been able to relate the genetic differences found within the different levels of cytokines in breeds described as resistant or susceptible to the disease.

The aim of this work is to analyze the relationship between the cytokine's serum levels and the genomic profiles in two canine breeds, Ibizan hound (resistant canine breed model) and Boxer (susceptible canine breed model).

2. Materials and Methods

2.1. Ethics Approval

The experiments involving animals were conducted according to the Declaration of Helsinki ethical principles and approved by the Animal Experimentation Ethics Committee of the Universidad Cardenal Herrera CEU, with code 2020/VSC/PEA/0216.

2.2. Animals and epidemiological data

Information of thirty-one Boxer and twenty-eight Ibizan hound dogs was recorded from animals living in the Valencia Community (Eastern Spain, Mediterranean region). Data and samples were recovered from October 2021 to June 2022. Apparently healthy

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dogs were tested for anti-*Leishmania* specific immunoglobulin G (IgG) antibodies by indirect immunofluorescent antibody test (IFAT) (MegaFLUO® LEISH, Megacor Diagnostik GmbH, Hörbranz, Austria). Only animals with IFAT titre <1/80 were considered seronegative [30] and included in this study. The epidemiological data of animals is shown in table 1.

Table 1. Epidemiological data of animals analyzed.

		No. of dogs (%)	
Variable	**Categories**	**Boxer**	**Ibizan hound**
Gender	Male	17 (54.84)	17 (60.71)
	Female	14 (45.16)	11 (39.29)
Age	Puppy (<1 year)	4 (12.90)	2 (7.14)
	Young (1 to 5 years)	10 (32.26)	6 (21.43)
	Adult (5 to 10 years)	14 (45.16)	9 (32.14)
	Elder (>10 years)	3 (9.68)	11 (39.29)
Diet	Only Commercial food	28 (90.32)	23 (82.14)
	Home prepared/raw food consumption	3 (9.68)	5 (17.86)
Overall		**31 (100.00)**	**28 (100.00)**

2.3. Samples collection and cytokines levels

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. The whole blood samples were used to DNA isolation before 24h to recovery.

The IL-2, IL-6, IL-8, IFN-γ (Canine IL-2 ELISA kit, Canine IL-6 ELISA kit, Canine IL-8 ELISA kit, and Canine IFN-γ ELISA kit, Invitrogen, respectively), and IL-18 (Canine IL-18 ELISA kit, Mybiosource) levels were measured in serum samples by commercial kit of ELISA method following the manufacturer recommendations. In brief, a 100 µl of serum was used for the analysis with the sandwich-ELISA. The microplate has been pre-coated with an antibody specific to cytokines. The sample is added to the microplate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for each cytokine and Avidin-Horseradish peroxidase (HRP) conjugate are added successively to each microplate well and incubated. Free components are washed away. The substrate solution is 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. DNA extraction and whole genome analysis

Genomic DNA (gDNA) from samples was isolated using a QIAamp DNA Blood Kit following the manufacturer's protocol (QIAamp; Qiagen, Hilden, Germany). DNA was quantified using the Glomax® Discover Fluorimeter and the QuantiFluor® dsDNA kit (Promega, Madison, WI, USA). gDNA concentrations for all samples were a minimum of 50 ng/µL. DNA samples were whole-genome amplified for 20-24 h at 37ºC, fragmented, precipitated and resuspended in an appropriate hybridization buffer.

Forty-eight samples (twenty-four Boxer and twenty-four Ibizan hound) were genotyped using the CanineHD DNA Analysis BeadChip WG-440-1001 (Illumina, Inc., San Diego, CA, USA) and hybridized on the prepared BeadChips for 16-24 h at 48ºC. Following the hybridization, nonspecifically hybridized samples were removed by washing, while the remaining specifically hybridized loci were processed for the single-base extension reaction, stained, and imaged on an Illumina iScan Reader. GenomeStudio 2.0.5 (Illumina Inc., San Diego, CA, USA) was used to process data generated from the iScan system for

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subsequent analysis, according to manufacturer guidelines. Intensity data was loaded into the Genotyping Module to primary data analysis, including raw data normalization, clustering and genotype calling. SNPs on sexual chromosomes and with a call rate <95% were discarded using PLINK v1.90b6.22 [31]. The final data set includes 165,480 mapped positions in samples with a mean genotyping rate of 0.988.

2.5. Statistical analysis

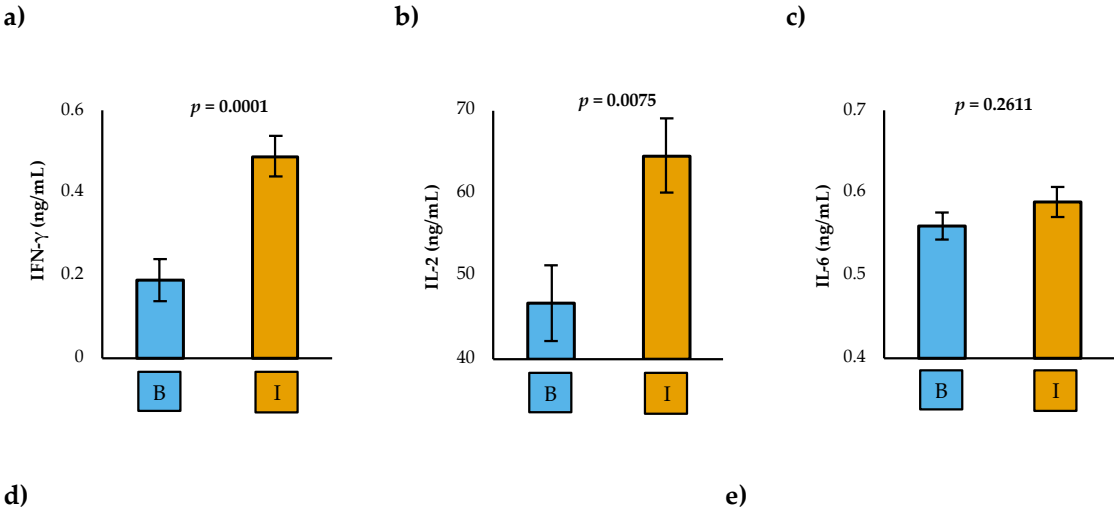
Serum levels of cytokines were analyzed using the general linear model procedure (PROC GLM) of the statistical package SAS (North Carolina State University, USA), after normality and homoscedasticity were tested by Shapiro-Wilks and Levene tests, respectively. The model was carried out with sex, age, and breed as fixed effects. Pearson's correlations between cytokine levels were carried out. The statistical significance was set at p-value < 0.05.

Polymorphisms included in each cytokine gene were selected from those genotyped according to the mapping information of the *Canis lupus familiaris* genome assembly CanFam3.1. Upstream and downstream regions (25kb) were added to each cytokine gene to include possible regulatory regions in the haplotype analysis (supplementary table 1). PLINK v1.90b6.22 was used to extract variants from the selected genome regions, according to the mapping information of the *Canis lupus familiaris* genome assembly CanFam3.1. The rsID information was downloaded and annotated from the European Variation Archive EVA release 3 files corresponding to the CanFam3.1 assembly.

Haplotypes for each sample were inferred using haplo.stats version 1.8.9 package in Rstudio [32]. The software haplo.stats computes scores to evaluate the association of a trait with the inferred haplotypes when the linkage phase is unknown. We used the Haplo.glm extension of haplo.score for estimating the magnitude of individual haplotype effects within each cytokine. The haplotypes with an absolute frequency less than 5 were later dropped by setting the haplo.min.count parameter to 5 for the final analysis to only account for mayor haplotypes.

3. Results

Differences between cytokines levels were found between the two canine breeds, being IFN-γ, IL-2, and IL-18 levels higher in Ibizan hound than in Boxer, whereas IL-8 were lower in Ibizan hound than in Boxer. No statistical differences were found in IL-6 levels between breeds (figure 1).



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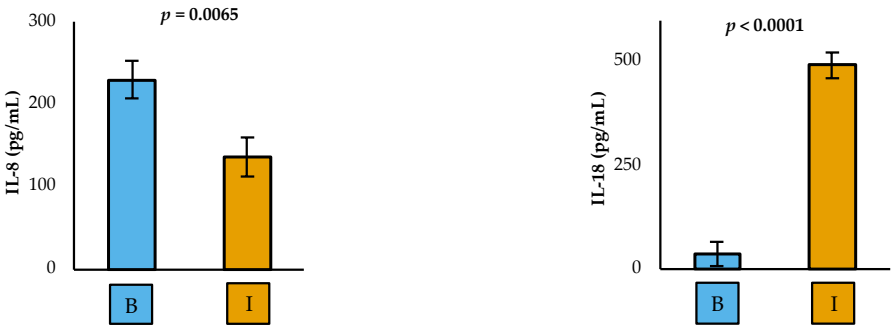

Figure 1. Serum levels of cytokines in Boxer (B) and Ibizan Hound (I). a) Interferon gamma (IFN-γ), b) Interleukin 2 (IL-2), c) Interleukin 6 (IL-6), d) Interleukin 8 (IL-8), and e) Interleukin 18 (IL-18). Squares represent LS means values for two breeds, and vertical lines represent standard deviation. 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.)

Mean levels of IFN-γ, IL-2, and IL-18 in Boxer dogs are 0.19 ± 0.05 ng/mL, 46.70 ± 4.54 ng/mL, and 36.37 ± 30.59 pg/mL respectively. In contrast, Ibizan hound dogs present 0.49 ± 0.05 ng/mL, 64.55 ± 4.54 ng/mL, and 492.10 ± 31.18 pg/mL, respectively. Related to IL-8, the values in Boxer dogs are higher than Ibizan hound dogs, being 230.04 ± 23.11 and 136.33 ± 23.55 pg/mL, respectively (table 2).

Table 2. Serum levels of cytokines analyzed in Boxer and Ibizan hound. Values for IL-8 and IL-18 are expressed in pg/mL, and for IFN-γ, IL-2, and IL-6 are expressed in ng/mL.

Cytokine[1]	Boxer[2] (LSMEAN ± SD)	Ibizan hound[2] (LSMEAN ± SD)	Mean square	F value	*p*-value
IFN-γ	0.19 ± 0.05	0.49 ± 0.05	1.14	16.95	**0.0001**
IL-2	46.70 ± 4.54	64.55 ± 4.54	4460.00	7.72	**0.0075**
IL-6	0.56 ± 0.09	0.59 ± 0.08	0.01	1.29	0.2611
IL-8	230.04 ± 23.11	136.33 ± 23.55	116315.75	8.06	**0.0065**
IL-18	36.37 ± 30.59	492.10 ± 31.18	2750907.87	108.85	**<0.0001**

[1]IFN: interferon; IL: interleukin.
[2]Serum levels of IFN-γ, IL-2 and IL-6 were measured in ng/mL, whereas serum levels of IL-8 and IL-18 were measured in pg/mL.

According to the calculated effects of haplotypes-serum levels interactions, resumed in the supplementary table 2, there were fifteen haplotypes that presented a statistically significant effect on the cytokine's serum levels. However, those showing a relative frequency of less than 50% in a specific breed were not considered in the forward discussion, as their effects are very limited to a few animals. Being those haplotypes involved in several *IL8* haplotypes, which are related to IL-8 and IL-18 serum levels. Therefore, according to the criteria, there are two haplotypes, *IL6* (CGAAG) and *IFNG* (GCA), that present an extended effect on the IL-2, IL-18 and IFN-γ serum values (figure 2).

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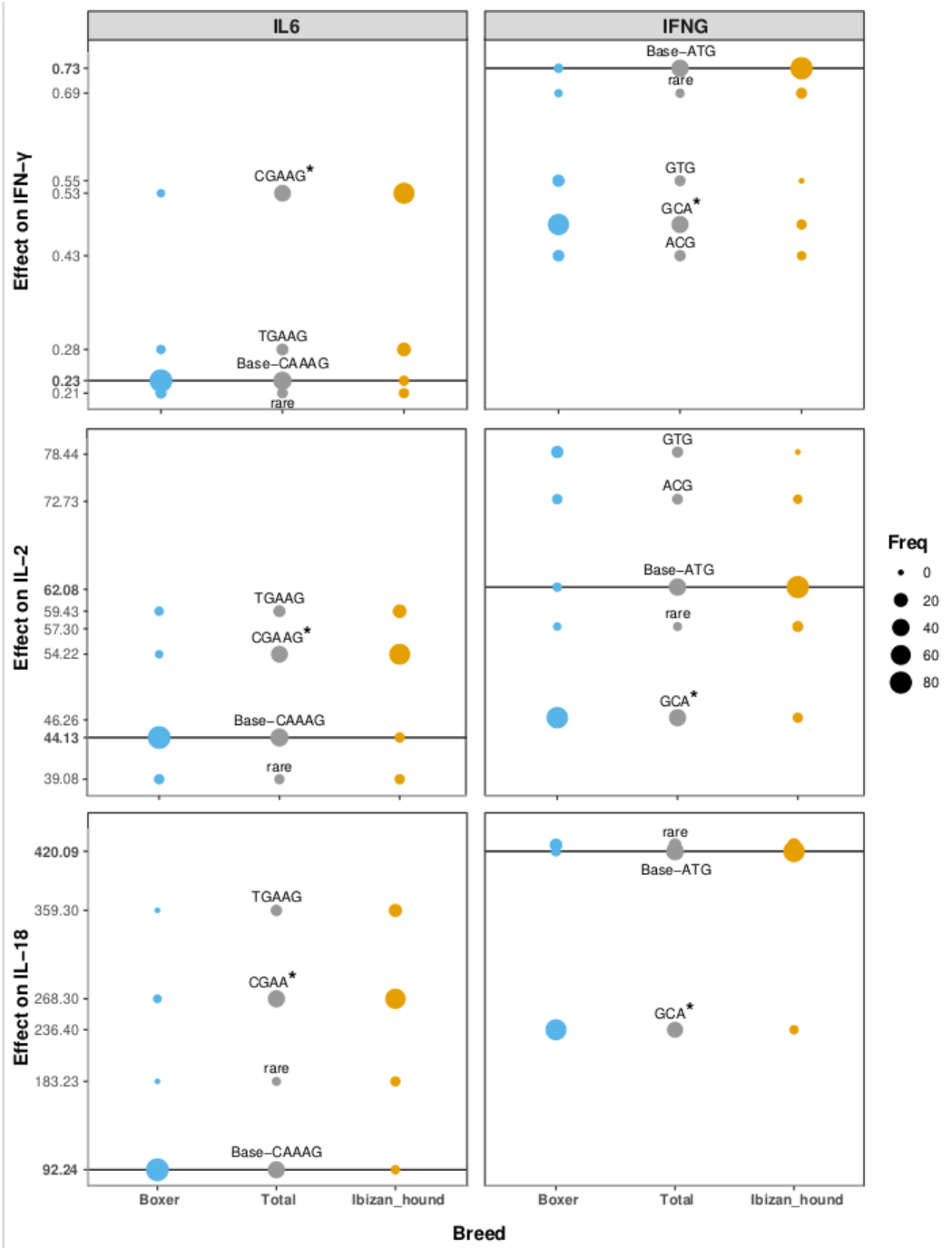

Figure 2. Effects of cytokines haplotype (*IL6*, *IFNG*)-serum levels (IFN-γ, IL-2, IL-18) interactions. Dots represent the expected serum values for each cytokine haplotype (Intercept basal haplotype values are signaled with a solid line), sizes are according to their relative frequencies, total and breed specific. Those statistically significant haplotypes are indicated with an *.

On the one hand, the *IL6*-CGAAG haplotype, compared against the reference haplogroup *IL6*-CAAAG (being the most frequent haplogroup > 40%), increases the IL-2, IL-8 and IFN-γ serum levels. Having a single copy of the *IL6*-CGAAG haplotype increases the value of the IL-2 in serum by 10.1 ($p < 0.05$); increases the value of IL-18 by 176.1 ($p < 0.001$) and increases the value of IFN-γ by 0.3 ($p < 0.01$) when compared to a dog being homozygous for the reference haplogroup. The *IL6*-CAAAG allele is almost exclusive of the Ibizan hound breed dogs with frequencies above 64% in the *IL2*, *IL8* and *IFNG* computed interactions.

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On the other hand, the *IFNG*-GCA haplotype that was compared against the reference haplogroup *IFNG*-ATG (>40%), decreases the IL-2, IL-18 and IFN-γ serum levels. Having a single copy of the *IFNG*-GCA haplotype decreases the value of the IL-2 in serum by 15.8 ($p < 0.001$); decreases the value of IL-18 by 183.7 ($p < 0.001$) and decreases the value of IFN-γ by 0.2 ($p < 0.05$) when compared to a dog being homozygous for the reference haplogroup. The *IL6*-CAAAG allele is almost exclusive of the Ibizan hound breed dogs with frequencies above 68.5% meanwhile *IFNG*-GCA is almost exclusive of the boxer breed dogs (>70%) in the *IL2*, *IL8* and *IFNG* computed interactions (table 3).

Table 3. Statistically significant effects of cytokines haplotype-serum interactions. Total haplotype frequencies of *IL6* (CGAAG) and *IFNG* (GCA) haplotypes are shown along with breed specific values. Coefficients summarized the estimated magnitude of individual haplotype effects.

Cytokine serum level affected[1]	Gen	Haplotype[2]	Total Freq.	Boxer Freq.	Ibizan hound Freq.	Coef.	*p*-value
IFN-γ	*IL6*	CGAAG*	35.6	2.1 (n=24)	68.8 (n=24)	0.29902	**0.004IL**
	IFNG	GCA**	38.3	70.8 (n=24)	6.3 (n=24)	-0.24964	**0.027**
IL-2	*IL6*	CGAAG*	36.5	2.2 (n=23)	68.7 (n=24)	10.085	**0.027**
	IFNG	GCA**	39.1	73.9 (n=23)	6.2 (n=24)	-15.8204	**< 0.001**
IL-18	*IL6*	CGAAG*	43.1	3.1 (n=16)	70.5 (n=22)	176.056	**< 0.001**
	IFNG	GCA**	35.1	78.1 (n=16)	4.5 (n=22)	-183.6948	**< 0.001**

[1]IFN: interferon; IL: interleukin
[2]*CGAAG: rs8888481, rs22301556, rs22303007, rs22370192, rs22370759; **GCA: rs22078594, rs22078627, rs22093388.

4. Discussion

The results of the present work show fifteen haplotypes within genes encoding cytokines that correlated with serum cytokines serum levels and with significant effect in apparently healthy dogs of the two considered canine breeds. Two of them, located in *IFNG* and *IL6* presented a great effect on the IFN-γ, IL-2 and IL-18 measured serum levels. The *IL6*-CGAAG increases the IFN-γ, IL-2 and IL-18, whereas the *IFNG*-GCA haplotype decreases it. The frequency of these two haplotypes differs between the two canine breeds analyzed, so *IL6*-CGAAG and *IFNG*-GCA haplotypes present high frequency in Ibizan Hound and in Boxer, respectively. According to these results, the Ibizan Hound dogs present higher serum levels of IFN-γ, IL-2 and IL-18 compared to Boxer dogs.

IFN-γ plays a relevant role in macrophages activation against *Leishmania* infection via NO [33–35]. When IFN- γ binds its receptor on the cell membrane of macrophages, the JAK-STA-1 pathways is activated, inducing IFN-γ stimulated genes [34]. Furthermore, several studies indicate that IFN-γ regulates the transcriptional mechanisms by alternative splicing and altering the microRNAs and lncRNAs expression [36]. Regarding *L. infantum*, the control of infection requires the activation of T helper 1 (Th-1) cells, which increases the IFN-γ and IL-2 serum levels [37,38]. The production of these cytokines was correlated with resistance to disease, so IFN-γ has been proposed as biomarker for immune monitoring in canine leishmaniasis [17,39,40], and IL-2 expression was negatively correlated with splenic parasite loads in infected dogs [41]. The IL-18, known as IFN-γ inducing factor, increases the production of this interferon by T cells and has a relevant role in the defense against visceral leishmaniasis [42,43]. According to that, these three cytokines (IFN-γ, IL-2 and IL-18) present high levels in Ibizan Hound dogs, which have a natural resistance against canine leishmaniasis [39,44–46]. Our results indicate that these elevated levels are correlated with *IL6*-CGAAG haplotype which is found with a higher

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frequency in the Ibizan Hound dogs compared to Boxer ones. Different haplotypes of *IL6* gene or *IL6R* genes have been related to IL-6 and other cytokines serum levels in humans, including IL-2, IL-8 and IL-18 cytokines [47–50], and with the severity or protective effect of the infectious disease, including parasitic diseases. For example, Mendonça et al. (2014), Sortica et al. (2014) and Wujcicka et al. (2015) founded *IL6* gene haplotypes related to the severity of the malaria disease and *Toxoplasma gondii* infection [51–53], whereas Chen et al. (2021) showed several *IL6* haplotypes with protective effect against COVID-19 infection [54]. According to the results of Yang et al. (2022) in human, our results show a correlation between *IL6* haplotype and serum levels of IL-18, in addition to finding an association with high levels of other cytokines as IFN-γ and IL-2, all of them related to protective effect against *L. infantum* infection.

On the contrary, Boxer dogs present higher frequency of *IFNG*-GCA haplotype which are related to low levels of IFN-γ, IL-2 and IL-18. Correlations between *IFNG* gene haplotypes and low level of cytokines have been previously detected in humans. In fact, da Silva et al. (2020) demonstrated a correlation between single haplotype of *IFNG* and low levels of IFN-γ, associated with the susceptibility to leishmaniasis [55]. According to these authors, the correlation between *IFNG*-GCA haplotype and the low levels of IFN-γ was also detected, together with low levels of other cytokines with a protective effect in *Leishmania* infection, as the IL-2 and IL-18. The IL-2 cytokine is secreted by the Th1 cells and stimulated the production of IFN-γ [56]. In fact, treatment of exogenous IL-2 in mice reduced parasitic load, increasing the *IFNG* expression [57]. Moreover, the IL-18 cytokine presents a protective effect against visceral leishmaniasis produced by *L. infantum* infection in humans [58], inducing IFN-γ and leading the production of Th1 responses and NK cells [59].

Although studies correlating haplotypes of *IFNG* gene and cytokines levels and/or susceptibility to infectious disease have not yet been carried out in dog, several studies described different haplotypes in this gene to be related to the susceptibility or resistance to infectious diseases in humans, including parasitic infection diseases. For example, some authors related *IFNG* haplotypes with the susceptibility against virus infection as hepatitis B virus [60], T-lymphotropic virus type 1 infection [61], or tuberculosis [62,63], against bacterial infection as brucellosis [64], and against parasitic infection as malaria [65,66]. Related to leishmaniasis, Kalani et al. (2019) found several haplotypes in *IFNG* gene related to susceptibility and resistance to visceral leishmaniasis in Iran [67].

Finally, our results (supplementary table 2) suggest a moderate effect of *IL8* haplotypes in IL-8 and IL-18 serum levels, which could have relationship between resistance or susceptibility to disease. Several haplotypes of *IL8* gene have been related to susceptibility and severity of different infectious diseases such as tuberculosis [68], syncytial virus disease [69], and hepatitis B virus [70]. In fact, several studies have demonstrated that some *IL8* haplotypes are related to the susceptibility of infectious diseases, increasing the percentage of ROS-producing monocyte-derived macrophages [71] and increasing the influx of neutrophils in inflammatory lesions [72]. However, the relationship between *IL8* haplotypes and cytokines serum levels, or susceptibility to *Leishmania* infection is still unknown. The limited number of dogs included in our study with these haplotypes difficult the obtention of determinant results. More studies related to these haplotypes and their effect on the severity of diseases are necessary to elucidate the molecular mechanisms of susceptibility and resistance to *L. infantum* in dogs and other mammals, including humans.

5. Conclusions

The Ibizan Hound is a canine breed which presented resistance against *L. infantum* infection. This resistance could be related to different cytokine serum levels profiles. This breed presents a lower prevalence of leishmaniasis than other canine breeds, and IFN-γ, IL-2 and IL-18 serum levels higher than other canine breeds such the Boxer, which has

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susceptibility to infection. Boxer dogs present higher levels of IL-8. In this study, haplotypes in the *IFNG* and *IL6* genes have been correlated to serum levels of IFN-γ, IL-2 and IL-18, and moderate effect on *IL8* haplotype correlated to IL-8 and IL-18 serum levels has been found. The results indicate that the resistance to *L. infantum* infection could be a consequence of certain haplotypes with a high frequency in the Ibizan Hound dog breed, while susceptibility to the disease would be related to other specific haplotypes, with high frequency in Boxer. Future studies will be necessary to elucidate the specific biological function of these haplotypes, its relationship with cytokine expression and regulation, and with different stages of disease in dogs and other mammals, including humans.

Supplementary Materials: Table S1: Analyzed polymorphism in the cytokines genes, including the identification number (rsID), chromosomes (Chr) and genomic position (Pos), according to CanFam3.1 assembly.; Table S2: Cytokines haplotype-serum interactions. Total haplotype frequencies are shown along with breed specific values. Coefficients summarized the estimated magnitude of individual haplotype effects. Coefficients values for base haplotypes correspond to the Intercept or constant value in the model.

Author Contributions: Conceptualization, L.A. and L.L.; methodology, L.A.; validation, L.A., P.R-G. and L.L.; formal analysis, L.A. and P-J. M-G.; data curation, P-J.M-G. and L.L.; writing—original draft preparation, L.A. and L.L.; writing—review and editing, L.A., P. R-G., P-J. M-G. and L.L.; supervision, L.L.; project administration, L.L.; funding acquisition, L.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board 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.

Informed Consent Statement: Informed consent was obtained from the owner of the animals involved in the study.
Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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