Comparative Immunology, Microbiology and Infectious Diseases xxx (xxxx) 102232

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Adaptive and innate immune response of *Leishmania infantum* infection in Cirneco dell'Etna dog breed

Lola Martínez-Sáez [a], Annalisa Amato [b], Carmelo Cavallo [b], Pablo Jesús Marín-García [c], Luigi Liotta [b,*], Lola Llobat [a,*]

[a] Molecular Mechanisms of Zoonotic Diseases (MMOPS) Research Group, Departamento Producción y Sanidad Animal, Salud Pública y Ciencia y Tecnología de los Alimentos (PASAPTA), Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Valencia 46113, Spain
[b] Department of Veterinary Sciences, University of Messina, Messina 98168, Italy
[c] Departamento Producción y Sanidad Animal, Salud Pública y Ciencia y Tecnología de los Alimentos (PASAPTA), Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Valencia 46113, Spain

ARTICLE INFO

Keywords:
Cirneco dell'Etna
Cytokines
Dog
Innate immune response
Leishmania

ABSTRACT

Leishmania spp. are an intracellular protozoa present in many countries around the world. In Europe, both the parasite and the disease it causes, leishmaniasis, are endemic in the Mediterranean basin. Clinical signs and severity of disease are highly variable depending on the host in both humans and dogs, traditionally considered the main reservoir of the parasite. The reason for these differences is not known, but it has been speculated that some hosts present immune response, related to activation of Th1 and Th17, capable of controlling the spread of the parasite, and that these immune responses are related to the genetic background of the host. The Ibizan hound, an autochthonous canine breed of the Mediterranean basin, has been postulated as a breed resistant to infection, but other canine breeds evolutionarily close to it and native to this region have not been studied. One of them is the Cirneco dell'Etna, native to the island of Sicily in southern Italy. In this study, the immune response against *L. infantum* infection in this canine breed was analysed. The results showed that infected dogs of this breed present high levels of several cytokines related to Th1 and Th17 immune response, and significant correlation between serum levels of cytokines related to disease resistance. Further studies are necessary in this canine breed to determine the mechanisms of immune response and genetic background related to *L. infantum* infection control.

1. Introduction

Leishmaniasis is a parasitic disease caused by protozoa from the genus *Leishmania*. These parasitic protozoan are transmitted to vertebrate hosts, including humans through the bites of infected female phlebotomine sandflies [23]. Different species of *Leishmania* cause the diseases and are distributed around the world [1], with leishmaniasis being endemic in different regions worldwide, including Africa, Asia, America and Europe. In Europe, the disease is endemic mainly in the Mediterranean basin [42]. Several species of mammals are involved in transmission, although the dog has traditionally been considered the main reservoir of the parasite [26]. Like humans, dogs present different clinical signs and severity of disease [8], but the cause of these individual differences is not yet fully understood. It is well known that T cell-mediated immunity plays a fundamental role in controlling the disease and several cytokines such as interferon gamma (IFN-γ) have been related to vaccination response [33] and with clinical presentation and progression of disease [25]. Genetic background of the host has been demonstrated as a relevant factor in the development of disease. In fact, polymorphisms of canine genes such as *NRAMP1* [2] or MHC class II [34] have been related to development of disease. In addition, some canine breeds have different prevalence of the disease [14], and some breeds such as the Ibizan hound do not develop clinical signs [39]. Studies carried out in this canine breed showed relevant differences in serum cytokine levels compared with the Boxer breed, considered one of the most sensitive [3], and genomic studies have demonstrated that Ibizan hound dogs have relevant polymorphisms in several genes related to immune response control [4].

Phylogenetic studies have shown that Ibizan hound are genetically close to other autochthonous canine breeds of the Mediterranean basin, such as the Cirneco dell'Etna or Pharaoh hound breed [41]. Cirneco dell'Etna is a descendant from the hunting dogs of the era of the

* Corresponding authors.
E-mail addresses: luigi.liotta@unime.it (L. Liotta), maria.llobatbordes@uchceu.es (L. Llobat).

https://doi.org/10.1016/j.cimid.2024.102232
Received 29 July 2024; Received in revised form 21 August 2024; Accepted 27 August 2024
0147-9571/

pharaohs in Ancient Egypt, like the Ibizan hound [10,32]. However, studies related to immune response in other possible resistant canine breeds such as Cirneco dell'Etna or Pharaoh hound are scarce.

The aim of this study is to analyse the cytokine profile in serum of Cirneco dell'Etna breed and crossbred dogs (mixed breed dogs), and its relation to immune response against *L. infantum* infection.

2. Material and methods

2.1. Animals and epidemiological data

A total of 48 dogs were studied, consisting of 18 animals living in the Valencia Community (Eastern Spain, Mediterranean region) and 30 in Catania province (Eastern Italy, Mediterranean region), from October to December 2023. Written informed consent was obtained from the owners of all participant dogs. Epidemiological data on age, sex, breed, vaccination status and clinical signs were collected. None of the animals received repellents and only dogs apparently healthy (without clinical signs compatible with *Leishmania* infection according to Solano-Gallego et al. [40]) were included in the study. Ages were grouped as young (from 1 to 5 years), adult (from 5 to 10 years) and elder (more than 10 years) [6]. According to canine breed, animals were divided into two groups, crossbred and Cirneco dell'Etna dogs with full known pedigree.

2.2. Sample collection and Leishmania infantum infection diagnostic

Ten millilitres of whole blood were taken by jugular venipuncture using two Vacutainer tubes, one with EDTA total DNA extraction and another without anticoagulant. The samples were kept at room temperature for 10 min and total DNA isolation was carried out within 24 h of whole blood extraction. Tubes without anticoagulant were centrifuged at 3000 rpm for 10 min and serum was separated and transferred into cryovials and stored at − 80 ºC until the laboratory analysis was carried out.

Serum samples were tested for anti-*Leishmania* specific immunoglobulin G (IgG) antibodies by indirect immunofluorescent antibody test (IFAT) (MegaFLUO® LEISH, Megacor Diagnostik GmbH, Hörbranz, Austria) and only samples with IFAT title > 1/80 were considered seropositive [29]. To confirm *L. infantum* infection, total DNA was extracted using Invitrogen PureLinkTM Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and only samples with A260/A280 ratio > 1.8 were used. Quantitative PCR was performed by amplification of kinetoplast minicircle DNA following the protocol published previously [9]. Briefly, the primers used were 5'-GGCGTTCTGCGAAAACCG-3', 5'-AAAATGGCATTTTCGGGCC-3' and TaqMan probe 5'-FAM-TGGGTGCAGAAATCCCGTTCA-3', and the reaction was carried out in 20 μL reaction containing 0.3 μM of each primer, 0.25 μM of probe and 20 ng of DNA. The conditions were a first step of UNG step at 50 ºC during 150 s, second step of denaturation for 10 min at 95 ºC, and 40 cycles of denaturation at 95 ºC for 15 s, and annealing-polymerisation at 60 ºC for 35 s.

2.3. Serum cytokine level analysis

The IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12 (Canine IFN-γ ELISA kit, Canine IL-2 ELISA kit, Canine IL-6 ELISA kit, Canine IL-8 ELISA kit, Canine IL-10 ELISA kit and Canine IL-12 ELISA kit, AssayGenie, Dublin, Ireland) levels were measured in serum samples by commercial ELISA kit method following the manufacturer's recommendations. In brief, a 100 μl of serum was used for the analysis with sandwich-ELISA. The microplate was pre-coated with an antibody specific to cytokines. The sample were 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 were added successively to each microplate well and incubated. Free components were washed away. 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 analysis

The homogeneity of epidemiological data on the dogs included was evaluated by Fisher's test analysis, and normality and homoscedasticity of quantitative variables were checked by Shapiro-Wilks and Levène test, respectively. Serum levels of cytokines and epidemiological data were analysed using the General Linear Model procedure (PROC GLM) of the SAS statistical package (version 9.2, North Carolina State University, USA) for each cytokine. The model was carried out with sex, age, breed and *L. infantum* infection as fixed effects and pairwise interactions were included. Lineal regression and Pearson's correlation test and between serum cytokine levels was carried out. The statistical significance was set at p-value < 0.05.

3. Results

Of all animals included in this study, 19 of all animals were males (39.6 %), whereas 22 were young (45.8 %) and 20 dogs were adults (41.7 %). Regarding breed, 30 dogs were Cirneco dell'Etna breed (62.5 %), whereas 18 dogs were crossbred (37.5 %). Twenty-four of the animals were positive for *L. infantum* infection (50 %), but none of them presented clinical signs compatible with the disease (Table 1). 12 of the Cirneco dell'Etna dogs and 12 crossbred dogs were *L. infantum* positive (50 %).

As to serum cytokine levels in infected and non-infected dogs, cytokine levels differed depending on the dog breed, being higher the levels of IL-2 and IL-6 in crossbred than in Cirneco dell'Etna dogs in non-infected (Table 2) and higher levels of IFN-γ and IL-8 in crossbred than

Table 1
Epidemiological data of dogs included in the study.

Variable	Categories	No. of dogs (%)
Sex	Male	19 (39.58 %)
	Female	29 (60.42 %)
Age	Young (1–5 years)	22 (45.83 %)
	Adult (5–10 years)	20 (41.67 %)
	Elder (> 10 years)	6 (12.50 %)
Breed	Cirneco dell'Etna	30 (62.50 %)
	Crossbred	18 (37.50 %)
L. infantum infection	Yes	24 (50.00 %)
	No	24 (50.00 %)

Table 2
Cytokine serum levels in non-infected dogs depending on canine breed. IFN: interferon; IL: interleukin. Levels of cytokines show as mean ± SD. p-value < 0.05 was considered as significant difference.

Cytokine serum levels (pg/mL)	Canine Breed		p-value
	Cirneco dell'Etna	Crossbred	
IFN-γ	322.06 ± 541.09	368.69 ± 307.71	0.844
IL-2	40.73 ± 50.77	1963.45 ± 228.37	< **0.05**
IL-4	130.58 ± 81.40	69.26 ± 24.49	0.086
IL-6	87.52 ± 81.47	3743.53 ± 435.46	< **0.05**
IL-8	166.34 ± 268.14	189.44 ± 152.49	0.844
IL-10	233.97 ± 413.18	140.51 ± 76.76	0.592
IL-12	242.26 ± 465.77	141.63 ± 68.35	0.608

in Cirneco dell'Etna dogs in infected dogs (Table 3). A statistical effect was observed of age and *L. infantum* infection according to canine breed. In fact, interactions between breed and several epidemiological factors included were significant, so that, although in the crossbred animals there was no effect of age, sex, or *L. infantum* infection on levels of any of the cytokines analysed (Table 4), there was an effect in Cirneco dell'Etna dogs. In this canine breed, infected dogs presented higher levels of IL-2 and IL-6 than non-infected, whereas levels of other cytokines analysed were similar between these groups. Related to age, in Cirneco dell'Etna dogs IL-6 serum levels were higher in elder than young and adult dogs (Table 5). High variability was found in serum levels of cytokines evaluated, mainly in IFN-γ, IL-2, IL-6 and IL-12.

Linear regression between cytokines was also related to canine breed. Whereas in Cirneco dell'Etna dogs IL-12 serum levels had linear regression with all cytokines evaluated, in crossbred dogs this regression only remained for IL-2 and IL-6. Cirneco dell' Etna dogs also had linear regression between levels of IL-10 and IFN-γ and IL-8.

In all animals included, high correlation ($r > 0.9$) was found between IFN-γ and IL-8, between IL-2 and IL-6, and between IL-10 and IL-12 (Figs. 1d, 1h, and 1u for crossbred, and Figs. 2d, 2h and 2u for Cirneco dell'Etna breed). Unlike what was observed in crossbred dogs, Cirneco dell'Etna dogs also showed strong positive correlations ($r > 0.9$) between IFN-γ and IL-12, and between IL-8 and IL-12 (Figs. 2f and 2t).

4. Discussion

In this study, serum cytokine levels in Cirneco dell'Etna and crossbred dogs seropositive and seronegative for *L. infantum* infection were evaluated. Results showed an effect of infection in levels of IL-2 and IL-6 only in Cirneco dell'Etna dogs. In this canine breed, adult animals had higher serum levels of IL-6 than elder or young animals. These differences were not observed in the crossbred dogs. Lineal regression was observed between IL-12 serum levels and all cytokines analysed in Cirneco dell'Etna, but not in the crossbred dogs. Finally, a relationship between cytokines evaluated changed depending on canine breed too, showing high correlation between IFN-γ and IL-12, and between IL-8 and IL-12, not found in crossbred dogs.

Resistance to *Leishmania* spp. infection is dependent on the activation macrophages and T helper 1 (Th1) cells, which produce proinflammatory cytokines favouring elimination of the parasite. Anti-inflammatory cytokines produced by T helper 2 (Th2) cells have been linked to proliferation and dissemination of parasite and disease progression [38]. IL-2 is one of the proinflammatory cytokines and has a relevant role in the proliferation of Th1 cells, essential for the activation of the host macrophages, and is associated with an increase in IFN-γ production [27]. This cytokine can promote T cell proliferation, as well as Treg development and activation [36]. In fact, immunotherapy with IL-2 reduces the infection of *Leishmania* spp. both *in vitro* [15] and *in vivo* [30]. Therefore, high values of this cytokine favour the host's ability to eliminate the parasite. However, the role of IL-6 in the regulation of *Leishmania* spp. infection does not seem so clear. Whereas several studies reported high levels of IL-6 during the active phase of visceral leishmaniasis in human [13,16], other authors stated that IL-6 has a pleiotropic effect, so induces the Th17 cells generation and stimulates IL-17 production [20]. This production of proinflammatory cytokine IL-17 is correlated with resistance to *L. infantum* infection both in humans [28] and dogs [35], acting as a synergic effect with IFN-γ.

The results of our study indicate that, while in crossbred dogs the infection does not modify the levels of these two cytokines, in Cirneco dell'Etna dogs infected by *L. infantum* both cytokines had higher values than in uninfected animals. This suggests a different immune response to infection depending on the genetic background of the host in agreement with Krayem and Lipoldová [21]. Differential serum levels of cytokines according to canine breed have been found previously [24] and higher levels of IL-2 were observed in Ibizan hound dogs (resistant breed) than in Boxer dogs (susceptible breed) [22]. Several studies indi-

Table 3
Cytokine serum levels in infected dogs depending on canine breed. IFN: interferon; IL: interleukin. Levels of cytokines show as mean ± SD. *p*-value < 0.05 was considered as significant difference.

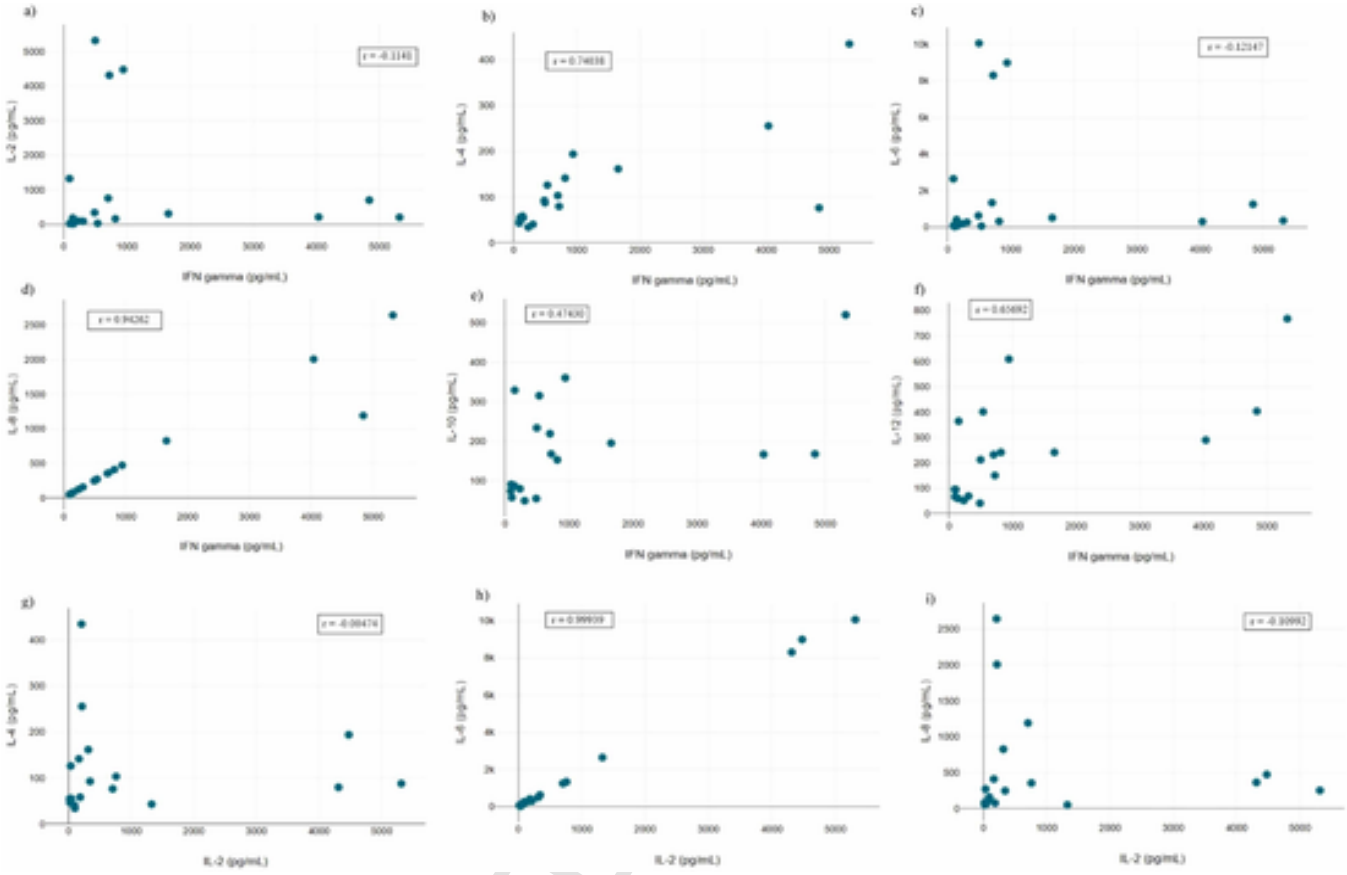
Cytokine serum levels (pg/mL)	Canine breed		p-value
	Cirneco dell'Etna	Crossbred	
IFN-γ	315.86 ± 297.12	1622.08 ± 194.07	< **0.05**
IL-2	137.32 ± 271.44	571.89 ± 124.31	0.249
IL-4	104.94 ± 102.67	139.09 ± 117.80	0.451
IL-6	294.39 ± 552.00	1110.16 ± 250.13	0.282
IL-8	163.26 ± 147.23	709.45 ± 83.42	< **0.05**
IL-10	144.87 ± 84.12	206.74 ±145.69	0.216
IL-12	154.48 ± 121.97	294.86 ± 230.96	0.076

Table 4
Cytokine serum levels in crossbred dogs. Different superscript within the same category shows statistical differences in cytokine serum levels. IFN: interferon; IL: interleukin. Serum levels of cytokines was show as mean ± SD.

Variable	Categories	IFN-γ (pg/mL)	IL-2 (pg/mL)	IL-4 (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IL-12 (pg/mL)
Sex	Male	1536.94 ± 208.36	627.96 ± 1248.74	117.91 ± 120.72	1184.95 ± 2402.14	658.06 ± 896.05	173.78 ± 142.90	230.63 ± 220.00
	Female	681.54 ± 523.30	1676.54 ± 2255.51	112.54 ± 60.01	3249.80 ± 4386.14	344.47 ± 259.32	201.76 ± 110.25	264.45 ± 189.35
Age	Adult	1433.45 ± 1850.07	872.1721.41	129.44 ± 105.60	1672.45 ± 3347.11	630.42 ± 794.14	198.02 ± 140.17	274.65 ± 219.79
	Elder	402.19 ± 357.61	1606.10 ± 1881.97	68.12 ± 28.62	3092.21 ± 3696.95	206.05 ± 177.22	137.91 ± 67.87	135.75 ± 73.06
L. infantum infection	Yes	1622.08 ± 1940.73	571.89 ± 1243.13	139.09 ± 114.80	1110.116 ± 2501.33	709.45 ± 834.23	206.74 ± 145.69	294.86 ± 230.96
	No	368.68 ± 307.71	1963.45 ± 2283.13	69.26 ± 24.90	3743.53 ± 4354.64	189.44 ± 152.49	140.51 ± 76.76	141.64 ± 68.35

Table 5
Cytokine serum levels in Cirneco dell'Etna dogs. Different superscript within the same category shows statistical differences in cytokine serum levels. IFN: interferon; IL: interleukin. Serum levels of cytokines was show as mean ± SD.

Variable	Categories	IFN-γ (pg/mL)	IL-2 (pg/mL)	IL-4 (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IL-12 (pg/mL)
Sex	Male	448.17 ± 616.18	161.24 ± 329.21	128.97 ± 78.13	323.48 ± 682.94	228.83 ± 305.35	292.03 ± 503.66	301.31± 547.61
	Female	272.82 ± 384.86	49.60 ± 65.36	117.26 ± 95.08	114.55 ± 109.50	141.93 ± 190.72	164.26 ± 235.61	172.91 ± 285.46
Age	Young	328.81 ± 497.55	46.78 ± 54.89	135.99 ± 96.23	99.00 ± 89.70[a]	169.68 ± 246.56	215.29 ± 375.61	221.83 ± 424.48
	Adult	343.97 ± 369.36	28.73 ± 37.28	83.91 ± 59.74	93.56 ± 69.00[a]	177.20 ± 183.04	168.38 ± 92.11	190.62 ± 133.26
	Elder	144.84 ± 35.62	589.79 ± 539.88	58.13 ± 1.09	1194.27 ± 1154.76[b]	78.52 ± 17.65	101.62 ± 40.28	95.31 ± 54.78
L. infantum infection	Yes	315.86 ± 297.12	137.32 ± 271.44[a]	104.94 ± 102.68	294.39 ± 552.00[a]	163.26 ± 147.23	144.87 ± 84.12	154.48 ± 121.97
	No	322.06 ± 541.09	40.73 ± 50.77[b]	130.68 ± 81.40	87.52 ± 81.47[b]	166.34 ± 268.14	233.97 ± 413.18	242.26 ± 465.77



(*caption on next page*)

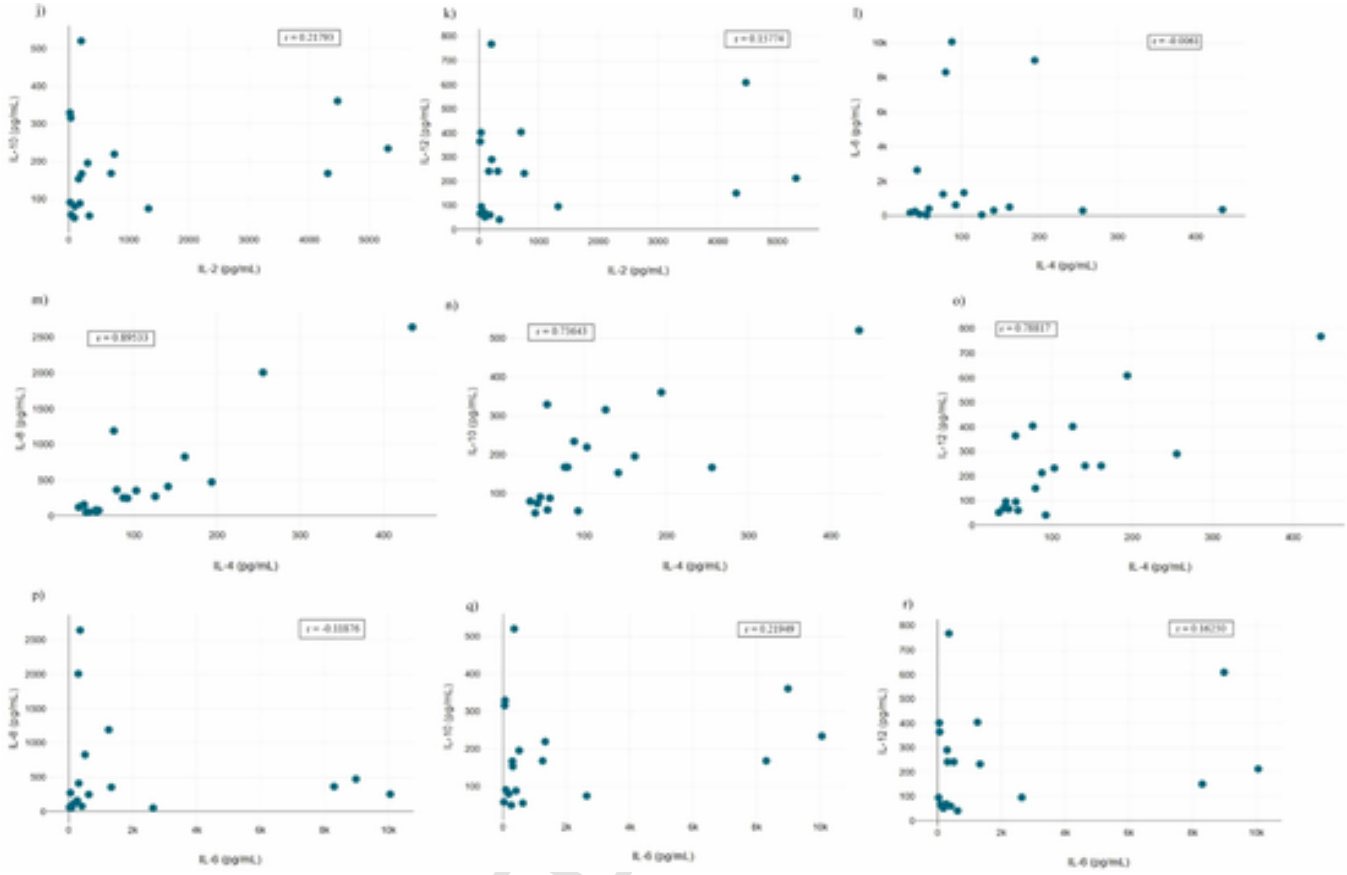


Fig. 1. Correlations found between serum levels of cytokines analyzed in crossbred dogs. IFN: interferon; IL: interleukin.; r: Pearson's correlation coefficient.

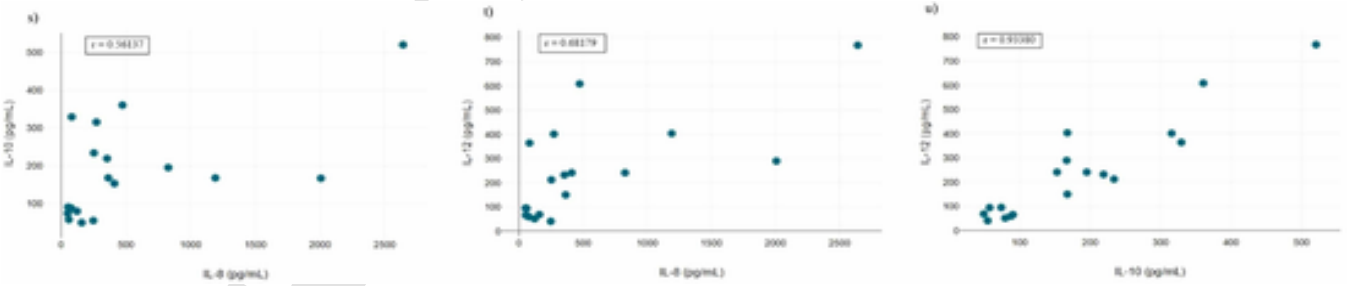


Fig. 1. (*continued*)

cated a relationship between these levels and some polymorphic variants in genes related to immunity and expression gene control both in Boxer [5] and Ibizan hound dogs [4]. Given the evolutionary closeness between Ibizan hound breed and Cirneco dell'Etna [10,32], genetic studies related to cytokine serum levels and genomic variants in this canine breed would be interesting to elucidate the mechanisms that confer a possible resistance to disease in Cirneco dell'Etna dogs and other related canine breeds. Cirneco dell'Etna dogs presented different serum levels of IL-6 according to age, these values being higher in adults than in young or elder dogs. This canine breed is mainly used for wild rabbit hunting (Federation Cynologique International), a practice for which adult animals are usually used. Hunting is one of the epidemiological factors associated with exposure to *L. infantum* in dogs [37], so the higher levels of IL-6 in these adult dogs could be related to greater exposure to the parasite.

High variability was found in cytokine serum levels, mainly in Cirneco dell'Etna dogs. This variability could be related not only to individual phenotypic and genetic variability, but also to the different populations of cells infected by *Leishmania* spp. In fact, Karagiannis et al. [18] has demonstrated that not only macrophages are parasitised, as other uncommon cell populations are related to *L. donovani* infection in human. In macrophages, the primary host cells for *Leishmania* parasites, a recent study showed the "sleepy macrophages" population and by Assay for transposase-accessible chromatin using sequencing (ATAC) technique demonstrated the heterogeneity post-infection with production of several cytokines, specifically IL-10 and IL-12, and the relationship between them in different macrophage populations during *L. major* infection [19]. In line with this, our study showed high correlation between serum levels of IL-10 and IL-12 in all animals included. Positive correlations between different serum cytokine levels have been reported in *L. guyanensis* infected humans [12], indicating complex and

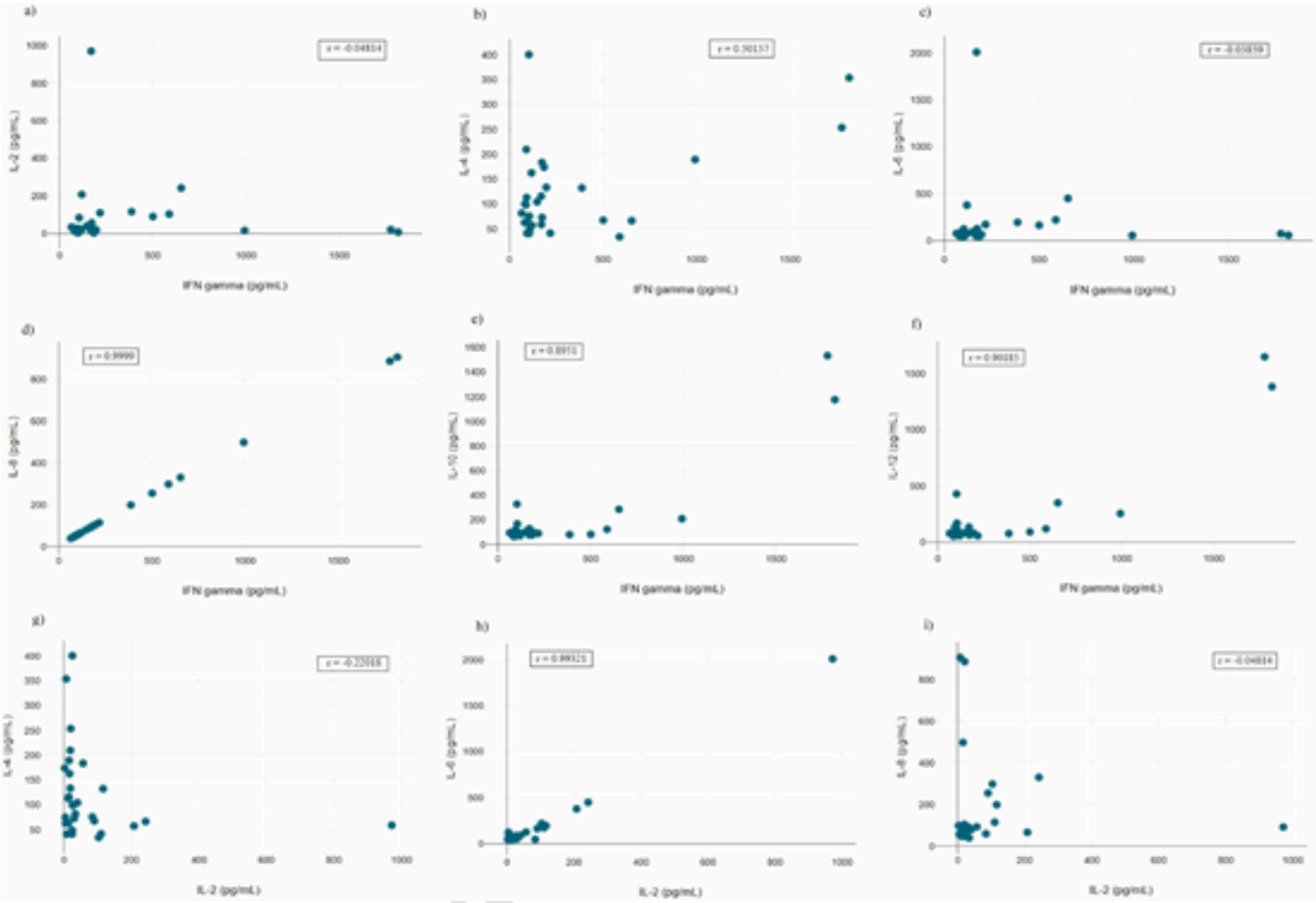


Fig. 2. Correlations found between serum levels of cytokines analyzed in Cirneco dell'Etna dogs. IFN: interferon; IL: interleukin.; r: Pearson's correlation coefficient.

still unknown relationships in the immune response related to *Leishmania* spp. infection control, both in resistant and susceptible individuals. In addition to the correlations observed in all the animals studied, Cirneco dell'Etna dogs in particular also presented high positive correlations between IL-12 with IFN-γ and IL-8 serum levels. Proinflammatory cytokine IL-12 has been studied as a possible candidate to develop Th1 immune response and to induce IFN-γ and facilitate NO production [11], and is required to maintain a Th1 response [31], whereas the relationship between IL-12 and IL-8 is still unclear, although the role of IL-8 as neutrophil recruitment is well known [7].

Control and regulation of serum cytokine levels are influenced by the *Leishmania* spp. infection in all organisms. Different expression patterns of genes encoding several cytokines, including IL-12, have been observed in human macrophages infected by *L. major* [17], which indicates complex regulation of infection control not only with cytokine levels, but also with regulatory mechanisms of gene expression. In fact, studies carried out in Ibizan hound showed polymorphisms in epigenetic regulatory genes [4], which could be related to an infection-resistant phenotype. Without a doubt, the immune response and its regulation is closely related to the genetic background of the host, and complex relationships between immunological factors play a relevant role in the appearance or not of clinical signs and the control of *Leishmania* spp. infection.

5. Conclusions

For the first time, immune response against *L. infantum* infection has been studied in Cirneco dell'Etna dogs, evolutionarily closely related to the Ibizan hound, known as a canine breed resistant to infection. Results indicate that the regulation of infection by cytokines is different in this canine breed from that in other dogs, and complex relationships between different cytokines are related to infection control, with a relevant role of cytokines IL-2, IL-6, and IL-12. Further studies to elucidate the mechanisms involved in infection resistance and genetic regulation of cytokines are necessary in this canine breed.

CRediT authorship contribution statement

Annalisa Amato: Methodology, Data curation. **Lola Martínez-Sáez:** Writing – original draft, Data curation. **Pablo Marín-García:** Methodology, Formal analysis. **Carmelo Cavallo:** Investigation, Data curation. **Lola Llobat:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Luigi Liotta:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The experimental protocol was approved by the Ethics Committee of Universidad Cardenal Herrera CEU (protocol code VCS/022/PEA/0279) and the Ethics Committee of the Messina University (protocol

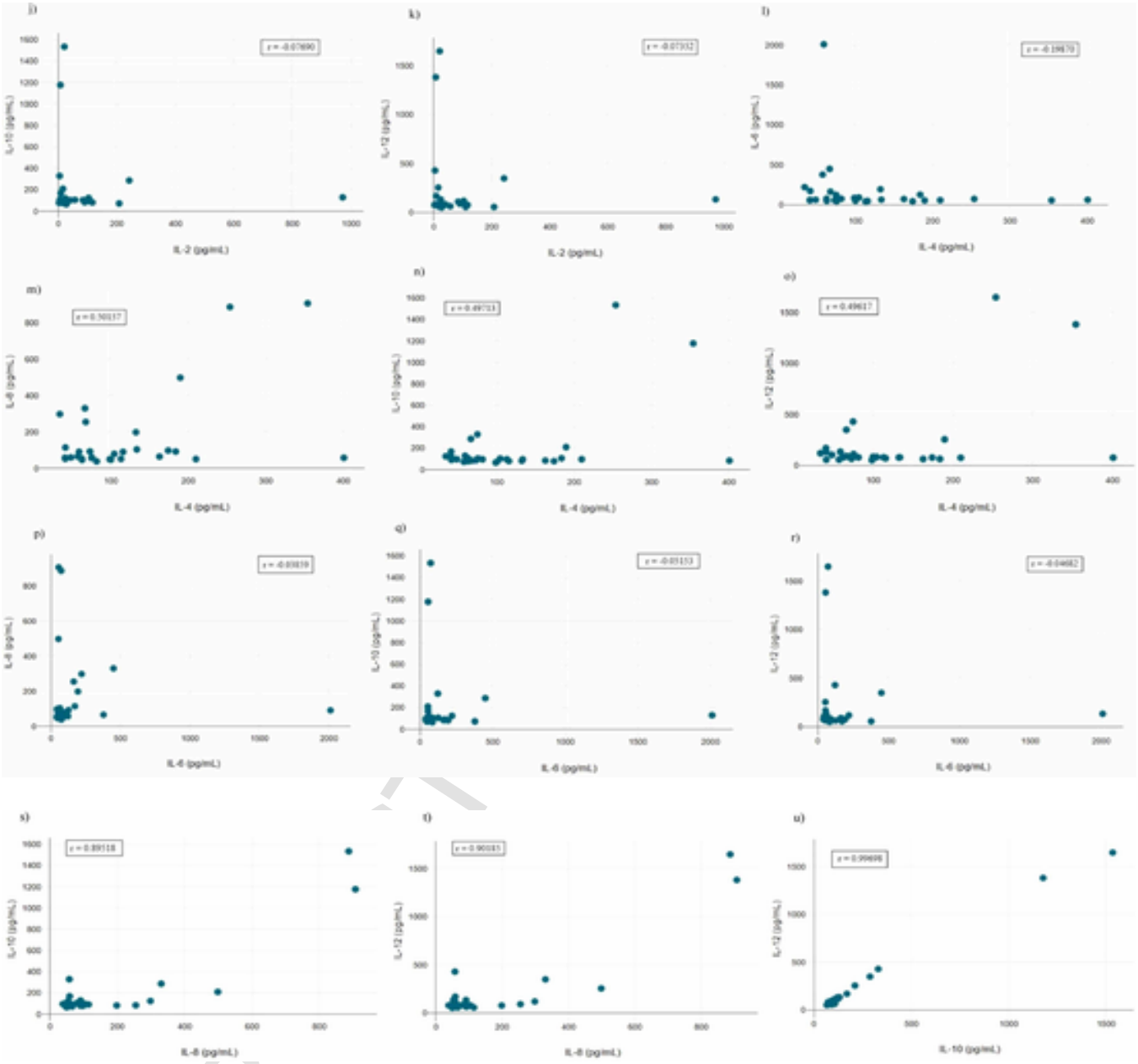


Fig. 2. (*continued*)

code 089/2022). The experimental protocols complied with the guidelines of the Ethic Committee. Blood samples were collected by the researchers (veterinarians) after getting informed consent from the owners of the horses. All efforts were made to minimize animal suffering during sample collection. Oral and written informed consent was obtained from all people who participated in the study.

Funding

This research was funded by Universidad Cardenal Herrera, Grant nos. IDOC23-01, INDI23-35 and GIR23-35. Lola Martínez-Sáez are supported by a Predoctoral Contract form the Universidad Cardenal Herrera CEU. The funders had no role in study design, data collection, and interpretation, or the decision to submit the work for publication.

Competing interests

The authors declare that they have no competing interests.

Declaration of Competing Interest

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

Acknowledgements

We are grateful to the University Cardenal Herrera CEU and the University of Messina.

Availability of data and materials

The data and material obtained are available to the researchers, with prior authorisation from the corresponding author upon reasonable request.

References

[1] M. Akhoundi, K. Kuhls, A. Cannet, J. Votýpka, P. Marty, P. Delaunay, D. Sereno, A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies, PLoS Negl. Trop. Dis. 10 (2016) e0004349, https://doi.org/10.1371/journal.pntd.0004349.

[2] L. Altet, O. Francino, L. Solano-Gallego, C. Renier, A. Sánchez, Mapping and sequencing of the canine NRAMP1 gene and identification of mutations in leishmaniasis-susceptible dogs, Infect. Immun. 70 (2002) 2763–2771, https://doi.org/10.1128/iai.70.6.2763-2771.2002.

[3] L. Álvarez, P.-J. Marín-García, L. Llobat, Serum levels and genetic variations of cytokines in two canine breeds (Ibizan hound and boxer) in the Mediterranean region, in terms of *Leishmania infantum* infection, Comp. Immunol. Microbiol. Infect. Dis. 90–91 (2022) 101908, https://doi.org/10.1016/j.cimid.2022.101908.

[4] L. Álvarez, P.-J. Marín-García, L. Llobat, Immunological and genomic characterization of Ibizan Hound dogs in an endemic *Leishmania infantum* region, Parasit. Vectors 15 (2022) 445, https://doi.org/10.1186/s13071-022-05504-3.

[5] L. Álvarez, P.-J. Marín-García, P. Rentero-Garrido, L. Llobat, Immune and genomic analysis of boxer dog breed and its relationship with *Leishmania infantum* infection, Vet. Sci. 9 (2022) 608, https://doi.org/10.3390/vetsci9110608.

[6] L. Álvarez, P.-J. Marín-García, P. Rentero-Garrido, C.P. Martinez-Jimenez, L. Llobat, Interleukin 6 and interferon gamma haplotypes are related to cytokine serum levels in dogs in an endemic *Leishmania infantum* region, Infect. Dis. Poverty 12 (2023) 9, https://doi.org/10.1186/s40249-023-01058-3.

[7] R. Badolato, D.L. Sacks, D. Savoia, T. Musso, *Leishmania major*: infection of human monocytes induces expression of IL-8 and MCAF, Exp. Parasitol. 82 (1996) 21–26, https://doi.org/10.1006/expr.1996.0003.

[8] G. Baneth, L. Solano-Gallego, Leishmaniasis, Vet. Clin. N. Am. Small Anim. Pract. 52 (2022) 1359–1375, https://doi.org/10.1016/j.cvsm.2022.06.012.

[9] G. Castelli, F. Bruno, S. Reale, S. Catanzaro, V. Valenza, F. Vitale, Molecular diagnosis of leishmaniasis: quantification of parasite load by a real-time PCR assay with high sensitivity, Pathogens 10 (2021) 865, https://doi.org/10.3390/pathogens10070865, ⟨https://www.fci.be/es/nomenclature/CIRNECO-DEL-ETNA-199.html⟩. (Accessed 23 May 2024).

[10] M. Cortellari, A. Bionda, A. Talenti, S. Ceccobelli, G. Attard, E. Lasagna, P. Crepaldi, L. Liotta, Genomic variability of Cirneco dell'Etna and the genetic distance with other dog breeds, Ital. J. Anim. Sci. 20 (2021) 304–314, https://doi.org/10.1080/1828051X.2021.1873076.

[11] A. D'Andrea, M. Rengaraju, N.M. Valiante, J. Chehimi, M. Kubin, M. Aste, S.H. Chan, M. Kobayashi, D. Young, E. Nickbarg, Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells, J. Exp. Med. 176 (1992) 1387–1398, https://doi.org/10.1084/jem.176.5.1387.

[12] T.G.R. de Mesquita, J. Junior, E.S. do, L.D.O. da Silva, G.A.V. Silva, F.J. de Araújo, S.K. Pinheiro, H.K.A. Kerr, L.S. da Silva, L.M. de Souza, S.A. de Almeida, K.L.G.D. Queiroz, J.L. de Souza, C.C. da Silva, H.D.G. Sequera, M.L.G. de Souza, A.N. Barbosa, G.S. Pontes, M.V. Guerra, F. de, R. Ramasawmy, Distinct plasma chemokines and cytokines signatures in *Leishmania guyanensis*-infected patients with cutaneous leishmaniasis, Front. Immunol. 13 (2022) 974051, https://doi.org/10.3389/fimmu.2022.974051.

[13] P.L. Dos Santos, F.A. de Oliveira, M.L.B. Santos, L.C.S. Cunha, M.T.B. Lino, M.F.S. de Oliveira, M.O.M. Bomfim, A.M. Silva, T.R. de Moura, A.R. de Jesus, M.S. Duthie, S.G. Reed, R.P. de Almeida, The severity of visceral leishmaniasis correlates with elevated levels of serum IL-6, IL-27 and sCD14, PLoS Negl. Trop. Dis. 10 (2016) e0004375, https://doi.org/10.1371/journal.pntd.0004375.

[14] M. Edo, P.J. Marín-García, L. Llobat, Is the prevalence of leishmania infantum linked to breeds in dogs? Characterization of seropositive dogs in Ibiza, Animals 11 (2021) 2579, https://doi.org/10.3390/ani11092579.

[15] Z. Eslami, M. Olivier, C.E. Tanner, Immunotherapy with IL-2-stimulated splenocytes reduces *in vitro* the level of *Leishmania donovani* infection in peritoneal macrophages, Int. J. Parasitol. 25 (1995) 975–981, https://doi.org/10.1016/0020-7519(94)00220-I.

[16] A.M. da C. Fievez, M.L. Silva-Freitas, A. de Q. Sousa, J.R. Santos-Oliveira, A.M. Da-Cruz, Lower levels of leptin are associated with severity parameters in visceral leishmaniasis patients, PLoS One 14 (2019) e0214413, https://doi.org/10.1371/journal.pone.0214413.

[17] K. Kalavi, O. Jorjani, M.A. Faghihi, S.J. Mowla, Cytokine gene expression alterations in human macrophages infected by *Leishmania major*, Cell J. 22 (2021) 476–481, https://doi.org/10.22074/cellj.2021.6524.

[18] K. Karagiannis, S. Gannavaram, C. Verma, T. Pacheco-Fernandez, P. Bhattacharya, H.L. Nakhasi, A.R. Satoskar, Dual-scRNA-seq analysis reveals rare and uncommon parasitized cell populations in chronic *L. donovani* infection, Cell Rep. 42 (2023) 113097, https://doi.org/10.1016/j.celrep.2023.113097.

[19] S. Khandibharad, S. Singh, Single-cell ATAC sequencing identifies sleepy macrophages during reciprocity of cytokines in *L. major* infection, Microbiol. Spectr. 12 (2024) e0347823, https://doi.org/10.1128/spectrum.03478-23.

[20] A. Kimura, T. Kishimoto, IL-6: regulator of Treg/Th17 balance, Eur. J. Immunol. 40 (2010) 1830–1835, https://doi.org/10.1002/eji.201040391.

[21] I. Krayem, M. Lipoldová, Role of host genetics and cytokines in *Leishmania* infection, Cytokine 147 (2021) 155244, https://doi.org/10.1016/j.cyto.2020.155244.

[22] M. Macià, P.-J. Marín-García, A.-E. Ahuir-Baraja, L. Llobat, Immunological profile of two canine breeds in an endemic region of *Leishmania infantum*, Vet. Parasitol. Reg. Stud. Rep. 40 (2023) 100861, https://doi.org/10.1016/j.vprsr.2023.100861.

[23] S. Mann, K. Frasca, S. Scherrer, A.F. Henao-Martínez, S. Newman, P. Ramanan, J.A. Suarez, A review of leishmaniasis: current knowledge and future directions, Curr. Trop. Med. Rep. 8 (2021) 121–132, https://doi.org/10.1007/s40475-021-00232-7.

[24] P.J. Marín-García, L. Llobat, Canine cytokines profile in an endemic region of *L. infantum*: related factors, Vet. Sci. 9 (2022) 305, https://doi.org/10.3390/vetsci9060305.

[25] P. Martínez-Orellana, D. Marí-Martorell, S. Montserrat-Sangrà, L. Ordeix, G. Baneth, L. Solano-Gallego, *Leishmania infantum*-specific IFN-γ production in stimulated blood from dogs with clinical leishmaniosis at diagnosis and during treatment, Vet. Parasitol. 248 (2017) 39–47, https://doi.org/10.1016/j.vetpar.2017.10.018.

[26] E. Montaner-Angoiti, L. Llobat, Is leishmaniasis the new emerging zoonosis in the world? Vet. Res. Commun. (2023), https://doi.org/10.1007/s11259-023-10171-5.

[27] H.W. Murray, G.D. Miralles, M.Y. Stoeckle, D.F. McDermott, Role and effect of IL-2 in experimental visceral leishmaniasis, J. Immunol. 151 (1993) 929–938.

[28] M.S.L. Nascimento, V. Carregaro, D.S. Lima-Júnior, D.L. Costa, B. Ryffel, M.S. Duthie, A. de Jesus, R.P. de Almeida, J.S. da Silva, Interleukin 17A acts synergistically with interferon γ to promote protection against *Leishmania infantum* infection, J. Infect. Dis. 211 (2015) 1015–1026, https://doi.org/10.1093/infdis/jiu531.

[29] A.I. Olías-Molero, M.J. Corral, M.D. Jiménez-Antón, J.M. Alunda, Early antibody response and clinical outcome in experimental canine leishmaniasis, Sci. Rep. 9 (2019) 18606, https://doi.org/10.1038/s41598-019-55087-w.

[30] M. Olivier, Z. Eslami, C.E. Tanner, IL-2-stimulated splenocytes reduce infections by *Leishmania donovani in vivo*, J. Parasitol. 82 (1996) 78–81.

[31] A.Y. Park, B.D. Hondowicz, P. Scott, IL-12 is required to maintain a Th1 response during *Leishmania major* infection, J. Immunol. 165 (2000) 896–902, https://doi.org/10.4049/jimmunol.165.2.896.

[32] F. Perini, I. Cardinali, S. Ceccobelli, A. Gruppetta, C.S. José, M. Cosenza, N. Musso, A. Martìnez, A.M. Abushady, L.V. Monteagudo, L. Liotta, H. Lancioni, G. Attard, E. Lasagna, Phylogeographic and population genetic structure of hound-like native dogs of the Mediterranean Basin, Res. Vet. Sci. 155 (2023) 103–114, https://doi.org/10.1016/j.rvsc.2023.01.010.

[33] M.M. Pompeu, C. Brodskyn, M.J. Teixeira, J. Clarêncio, J. Van Weyenberg, I.C. Coelho, S.A. Cardoso, A. Barral, M. Barral-Netto, Differences in gamma interferon production in vitro predict the pace of the in vivo response to *Leishmania amazonensis* in healthy volunteers, Infect. Immun. 69 (2001) 7453–7460, https://doi.org/10.1128/IAI.69.12.7453-7460.2001.

[34] R.J. Quinnell, L.J. Kennedy, A. Barnes, O. Courtenay, C. Dye, L.M. Garcez, M.-A. Shaw, S.D. Carter, W. Thomson, W.E.R. Ollier, Susceptibility to visceral leishmaniasis in the domestic dog is associated with MHC class II polymorphism, Immunogenetics 55 (2003) 23–28, https://doi.org/10.1007/s00251-003-0545-1.

[35] G.T. Rebech, G.L. Venturin, L.T. Siqueira Ito, J.P. Bragato, B.S. de Carvalho Fonseca, L.M. Melo, S.F. Costa, F. de Rezende Eugênio, P.S.P. Dos Santos, V.M.F. de Lima, PD-1 regulates leishmanicidal activity and IL-17 in dogs with leishmaniasis, Vet. Immunol. Immunopathol. 219 (2020) 109970, https://doi.org/10.1016/j.vetimm.2019.109970.

[36] Y. Rochman, R. Spolski, W.J. Leonard, New insights into the regulation of T cells by gamma(c) family cytokines, Nat. Rev. Immunol. 9 (2009) 480–490, https://doi.org/10.1038/nri2580.

[37] P. Rombolà, G. Barlozzari, A. Carvelli, M. Scarpulla, F. Iacoponi, G. Macrì, Seroprevalence and risk factors associated with exposure to *Leishmania infantum* in dogs, in an endemic Mediterranean region, PLoS One 16 (2021) e0244923, https://doi.org/10.1371/journal.pone.0244923.

[38] A. Saha, S. Roy, A. Ukil, Cytokines and signaling networks regulating disease outcomes in leishmaniasis, Infect. Immun. 90 (2022) e0024822, https://doi.org/10.1128/iai.00248-22.

[39] L. Solano-Gallego, J. Llull, G. Ramos, C. Riera, M. Arboix, J. Alberola, L. Ferrer, The Ibizian hound presents a predominantly cellular immune response against natural *Leishmania* infection, Vet. Parasitol. 90 (2000) 37–45, https://doi.org/10.1016/s0304-4017(00)00223-5.

[40] L. Solano-Gallego, G. Miró, A. Koutinas, L. Cardoso, M.G. Pennisi, L. Ferrer, P. Bourdeau, G. Oliva, G. Baneth, The LeishVet Group, null, LeishVet guidelines for the practical management of canine leishmaniosis, Parasit. Vectors 4 (2011) 86, https://doi.org/10.1186/1756-3305-4-86.

[41] A. Talenti, D.L. Dreger, S. Frattini, M. Polli, S. Marelli, A.C. Harris, L. Liotta, R. Cocco, A.N. Hogan, D. Bigi, R. Caniglia, H.G. Parker, G. Pagnacco, E.A. Ostrander, P. Crepaldi, Studies of modern Italian dog populations reveal multiple patterns for domestic breed evolution, Ecol. Evol. 8 (2018) 2911–2925, https://doi.org/10.1002/ece3.3842.

[42] E. Torres-Guerrero, M.R. Quintanilla-Cedillo, J. Ruiz-Esmenjaud, R. Arenas, Leishmaniasis: a review, 2017. ⟨https://doi.org/10.12688/f1000research.11120.1⟩.