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Research Article

Canine Leishmaniasis: The relation between the height of the DAT antibody titer and clinical, hematological and biochemical manifestations

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ABSTRACT

Background: CanL is a disease caused by the Protozoa *Leishmania infantum.* This disease can lead to several clinical signs and manifestations in the hematological analysis, urinalysis and biochemical profile. Some clinical signs are associated with the individual animal's immune response. The Direct Agglutination Test can be used to diagnose the disease by assessing the height of the antibody level against Leishmania. Several clinical staging systems for CanL exist and are currently being used to predict the severity, treatment and prognosis of the disease. The purpose of this study is to further investigate the correlation between the height of the antibody titer and clinical, hematological and biochemical manifestations.
Materials and methods: A retrospective study was conducted based on patient files of dogs with a DAT antibody result for CanL. 118 dogs were included in the study of which 80 dogs had a negative (≤1:40) and 38 dogs had a positive (>1:40) antibody titer. Of each dog, results of the anamnesis, the physical exam, the hematology and biochemical bloodwork were collected from their files. All dogs with a diagnose of CanL were retrospectively classified in three different clinical staging systems.
Results: Lymphadenomegaly, alopecia and crustae on the ears were the most common clinical signs found in dogs with CanL. The most frequent hematological and biochemical findings in these dogs, were a hypergammaglobulinemia, hypoalbuminemia and hyperproteinemia. Positive, untreated dogs showed a significant higher level of gamma-globulins and total proteins but a lower UPCR. A significant positive correlation between the height of the antibody level and the level of urea and a negative correlation between the height of the antibody level and the level of leucocytes was found. No correlation was found between the height of the antibody level and three different clinical staging systems.
Conclusion: The results of this study partly support the idea that DAT titers are related to the hematological and biochemical manifestations but reject the hypothesis that the DAT titer is correlated to clinical manifestations. Further studies are needed in order to further explore this possible correlation.

1. Introduction

Leishmania infantum causes Canine Leishmaniasis (CanL), a protozoal disease that is transmitted via phlebotomine sandflies. This parasite has a cycle in which the dog is bitten by a sandfly and this way infects the dogs with the infectious flagellated promastigote.[1] The dogs macrophages phagocytose the promastigote after which they develop intracellular to the amastigote and replicate. The amastigote then circulates through the blood and ends up at different visceral organs where it could cause inflammatory changes and substantial damage with a possible fatal end.

The disease is widely distributed in the Mediterranean areas but due to climate changes and dogs travelling, it is spreading in a northern direction from South-Europe.[2] There have been autonomous cases of Canine Leishmaniasis in Germany and Romania and different species of the Phlebotomine sandflies, that are suspected of transmitting Leishmaniasis, have been found in these countries.[3,4]

CanL can give a wide range of clinical presentations as it can cause damage to any organ and can vary from subclinical infection to life-threatening disease. Dogs can be asymptomatic or subclinical infected or show a wide range of general symptoms such as lymphadenopathy, weight loss, anorexia and exercise intolerance.[5] Some of the clinical signs are associated with the individual animal's immune response. The role of this protective immunity and the concurrent disease is very important. Infected dogs with clinical Leishmaniosis show a depressed cellular response but an activation of the humeral immunity system. Depending on the immune reaction of an infected dog, Th2-lymphocytes and IL-4 secretors cause a variable production of antibodies and cause a variation in disease susceptibility.[6]

Diagnosing CanL is therefore based on the amount of antibodies that are produced. A quantative serological technique is most commonly used to detect the specific serum antibodies: an enzyme-linked immunosorbent assay (ELISA), an immunofluorescence antibody test (IFAT) or a direct agglutination test (DAT). These techniques result in a relative concentration and a high antibody titer confirms an infection with Leishmania. However, quantitative serology does not differentiate between disease and asymptomatic infection: dogs show no clinical signs but do have an antibody response that can confirm the infection.[7] Both clinical and subclinical infected dogs show higher IgG2, IgA and IgE in the blood, but only IgG2 is positively correlated with clinical manifestations.[8] Contradictory, other studies only confirm a strong correlation between IgE and clinical manifestation.[9] Other diagnostic methods exist that can confirm the diagnosis when the antibody titer is low or doubtful. These methods include PCR, cytology and histopathology.[10]

The antibodies produced are soluble immune complexes and are deposited in organs and tissues. This is associated with severe clinical manifestations. These antibody-dependent symptomatic manifestations are therefore more specific for Leishmaniasis and include cutaneous problems like hyperkeratosis of the skin or nose, ulcerations, exfoliative dermatitis and pustular/papular dermatitis [11], but also renal dysfunction, epistaxis and ocular lesions. [5]

Besides clinical signs, there can be multiple but nonspecific changes in the hematological analysis, urinalysis and biochemical profile of the infected dog. First of all, parasite infection in the bone marrow and liver could show changes in the hematopoietic and hepatic function resulting in anemia, lymphopenia, leukocytosis and elevated liver enzymes.[12] Secondly, the immune response of symptomatic dogs presents a decreased level of albumin and an increased level of the globulin fraction (especially gamma-globulins), resulting in an inversion of the albumin/globulin ratio.[12–14] Also, changes can be seen in the renal function parameters, like urea and creatinine, when analyzing the urine and blood.[12,15] These laboratory abnormalities can be used to support the diagnosis, the staging of the disease, the follow-up examinations and help determine the prognosis.[7]

Staging CanL could also give more information on the management of the disease. Because Leishmaniasis can cause such a wide range of clinical signs, it is thought to be helpful to use a clinical classification system in order to determine a prognosis and treatment plan. Several classification systems for Leishmaniasis exist and are currently being used around the world. The two most widely used are The Leishvet staging system[7] and the Canine Leishmaniasis Working Group (CLWG) staging system[16] because both include prognosis information and therapeutic recommendations. Another useful classification system is established by Amusategui et al. (2003) which also includes the height of the antibody level as a criterion.[17]

Besides the clinical classification systems, the total antibody response could be a tool to predict the severity of the clinical course of the disease and therefore, could help establish the most suitable treatment plan for each patient. Research shows that dogs with severe clinical manifestations have a significant higher antibody titer and a higher quantity of Leishmania DNA in lymphnodes.[18] Thus, indicating a positive relationship between the height of the titer and clinical signs, but only in severe cases. This was also observed by another study who concluded a positive correlation between serological reactivity and the clinical status.[19] Proverbio et al. (2014) also concluded that dogs with the highest antibody titers have a higher mean clinical score, especially in severe forms of the disease.[20] They also found correlations between biochemical results and the height of the titer. Another study by Mélendez-Lazo et al. (2018) also found a difference in some parameters of the hematological and biochemistry results between dogs with a low, medium and high antibody level.[12] This also indicates a relation between the height of the antibody level and laboratory alternations. In contrast, another study found no correlation between the level of antibody titers and a reduction of the clinical signs after treatment.[21]

The purpose of this study is to further investigate this correlation between the height of the antibody titer and clinical, hematological and biochemical manifestations. Therefore, this research contains two research goals:

1. To determine if there is a difference between the clinical, hematological and biochemical manifestations of dogs with a positive and negative Leishmania antibody titer.
2. To determine if the height of the DAT antibody titer for Canine Leishmaniasis is correlated to clinical, hematological or biochemical manifestations.

2. Material and methods

2.1. Clinical data collection:

The files of dogs were included that had a tentative diagnosis of Leishmaniasis based on travel history and suggestive clinical signs. Dogs were classified as negative when the Direct Agglutination Test (DAT) result was ≤1:40 and positive when the DAT result was >1:40.

The following inclusion criteria were used:

- A complete medical file containing a DAT titer performed at the Utrecht University Veterinary Diagnostic Laboratory (UVDL), a detailed anamnesis, general physical examination, dermatological examination and the results of the laboratory blood and urine tests.
- Results of the anamnesis, physical examination, dermatological examination and laboratory blood and urine test must be collected within 7 days from obtaining the result of the DAT titer.
- All laboratory tests must be performed within a week before or after the DAT titer was determined.
- The medical file must be detailed enough to, retrospectively, confirm the diagnosis of Leishmaniasis.
- Dogs must be born in or have travelled to endemic areas.

The following exclusion criteria were used:

- Dogs who did not travel to endemic areas
- Dogs who had an incomplete medical file which lacked results of the anamnesis and/or physical examination.

The files used in this study must include a result from the Leishmania antibody titer by a DAT performed at the UVDL of which the positive sera are further serially diluted to establish the maximum reaction titer. The dogs were either presented at the University of Utrecht Clinic for Companion Animals (UUCCA) or at a local veterinary practice in the Netherlands between October 2019 and November 2020. Before October 2019, the positive sera were not diluted to establish this maximum reaction. The required data, including the results of the anamnesis, the physical exam, the hematology and biochemical bloodwork, was extracted from medical records in the veterinary software program Vetware or obtained from veterinary practices in The Netherlands. The data from the local veterinary practices was provided by the practices themselves after the owner signed an informed consent. (Appendix 1)

The following data was collected from these files:
Signalment: sex, breed and age at time of antibody titer
Anamnesis: clinical signs seen by the owner, history of the dog including country of origin and travel history, any form of treatments for Leishmaniosis.
Physical examination: respiratory rate, pulse rate, rectal temperature, color and aspect of mucosal membranes, palpation of the lymph nodes, detailed description of skin and/or nail lesions.
Hematology and biochemistry results: Hematocrit, reticulocytes, lymphocytes, leucocytes, neutrophils, monocytes, eosinophils, thrombocytes, protein specter (Alpha-1, Alpha-2, Beta-1, Beta-2 and gamma-globulins), total protein, albumin, creatinine and urea.
Urinary results: specific gravity, pH, protein, creatinine and urea-protein-creatinine ratio (UPCR).

A list of clinical signs was composed, including a wide range of clinical parameters most common of Leishmaniasis. Each dog's file was thoroughly read, and each parameter was scored in a binary fashion (yes or no) of either showing (scored 1) or not showing (scored 0) this particular clinical sign. The list of clinical signs included lymphadenopathy, decreased appetite, weight loss, decreased endurance, muscle atrophy, lameness, fever, alopecia, dry exfoliative dermatitis, papular/nodular dermatitis, ulcerative lesions skin, ulcerations on footpads, onychopathy, pustules, squamae, crustae on ears, crustae on body, erythema, hyperkeratosis, hyperpigmentation, depigmentation, pale mucous membranes, mucocutaneous ulcerative lesions, diarrhea, vomiting, polyuria and polydipsia, epistaxis, myositis, conjunctivitis, uveitis and splenomegaly.

2.2. Treatment

A difference was made between dogs that received treatment for their Leishmaniasis around the time the DAT titer was performed and dogs who did not. It is known that allopurinol has an influence on the clinical signs, laboratory blood results and antibody titer of a treated dog. Dogs who received Allopurinol for more than four weeks, were classified as "treated positive dogs". Dogs who received no treatment of received Allopurinol for less than four weeks, were classified as "untreated positive dogs". This difference results in included dogs being classified into:

- Negative group
- Positive group
    - Treated positive group
    - Untreated positive group

2.3. Assignment according to classification systems

The dogs were classified in the most appropriate category, using three different clinical staging systems: Amusategui et al. 2003, Leishvet group by Solano-Gallego et al. (2009) and the Canine Leishmaniosis Working Group (CLWG) by Paltrinieri et al. (2010). These clinical classification systems were chosen because of their clearly defined inclusion criteria and because most dogs could be classified in one of their stages. In some cases, the dogs could not be classified because they did not meet the criteria for classification in any possible way. These dogs were defined as "unclassified". A summary of the staging systems is shown in table 1.

2.4. Statistical analyses

To answer the first research aim, a Fisher's Exact test was performed to assess the difference in occurrence of the clinical signs between the positive and negative group. For most of the clinical signs, an odds ratio could be calculated. The Haldane correction method was used if any of the cell values would cause a division by zero error. The Mann-Whitney-U test was used to assess any statistically significant difference in the mean of the laboratory results between the untreated positive and negative dogs. As the treatment could have had an effect on the blood and urine laboratory results, this analysis was only performed with the group of untreated positive dogs. See Appendix 2 for a list of definitions.
To answer the second research aim, the Spearman's correlation was used on the data of the positive, untreated dogs to evaluate the degree of association between the DAT titer and hematological and biochemical manifestations and between the DAT titer and the classification systems. Again, only using the group of untreated positive dogs because treatment could influence both the blood and urine laboratory results and the height of the DAT titer.
All statistical analyses were performed using commercial software (IBM SPSS statistics, Version 26.0). All hematological and biochemical parameters were tested for normality using the Shapiro-Wilk test. Outliers were detected by histogram evaluation. Significance was set as $P < .05$

Study - staging system	**Clinical classification based on clinical signs**	**Further diagnostic testing**
Amusategui et al. 2003	1. Initial stage: asymptomatic or with mild, non-specific clinical signs. 2. Established disease: typical clinical signs of canine Leishmaniosis. 3. Advanced stage: severe organic complications (renal, hepatic, cardiac, etc.).	1. Slight dysproteinemia or a non-altered serum protein electrophoretogram, antibody titer 1/100 ≤ 1/800. 2. Dysproteinemia, antibody titer ≥ 1/400 3. Serious biochemical and haematological alterations; variable dysproteinemia and variable antibody titers.
Solano-Gallego et al. (2009) - LeishVet	1. Mild disease: mild clinical signs such as localized lymphadenomegaly and popular dermatitis. 2. Moderate disease: apart from signs listed in stage 1 may present: skin disorders, anorexia, weight loss, fever and epistaxis. 3. Severe disease: apart from signs listed in stages 1 and 2, may present signs originating from immune-complex lesions. 4. Very severe disease: dogs with clinical signs listed in stage 3, may present pulmonary thromboembolism.	1. Usually no clinicopathological abnormalities. 2. Low to high positive antibody levels. Clinicopathological abnormalities such as mild non-regenerative anemia, hyperglobulinemia, hypoalbuminemia, serum hyperviscosity syndrome. a. normal renal profile: creatinine <1.4 mg/dL; nonproteinuric UPC ,0,5 b. creatinine <1.4 mg/dL; UPC = 0,5-1 3. clinicopathological abnormalities listed in stage 2. CKD IRIS stage I with UPC > 1 or stage II (creatinine 1.4-2 mg/dL). 4. medium to high positive antibody levels. Clinicopathological abnormalities listed in stage 2, CKD IRIS stage III (creatinine 2-5 mg/dL). Nephrotic syndrome: marked proteinuria UPC>5 and end-stage renal disease.
Paltrinieri et al. (2010) – CLWG	1. Exposed 2. Infected: dogs are clinically normal or have signs associated with other diseases 3. Sick: one or more clinical signs common to leishmaniosis are present. Dogs without clincial signs but with laboratory alterations. 4. Severely sick: dogs with severe clinical illness. Concurrent problems that require immunosuppressive treatment; severe concomitant conditions; and clinical unresponsiveness to repeated courses of anti-Leishmania drugs. 5. A) sick unresponsive B) sick-early relapse	1. Negative cytologic, histologic, parasitological and molecular findings and low titer antibodies against *Leishmania spp.* 2. Dogs in which parasites have been detected through direct diagnostic methods and with los-titer antibodies against *Leishmania spp.* 3. Dogs with positive cytologic results regardless of serologic results and dogs with high antibody titers against *Leishmania spp.* Hematologic, biochemical and urinary alterations common to Leishmaniasis. 4. Evidence of proteinuric nephropathy or chronic renal failure.

Table 1 Three clinical classification systems and their criteria for dogs with CanL

3. Results

3.1. Descriptive analysis

In total, 128 dogs had a DAT titer result from the UVDL. One dog was yearly vaccinated with a leishmania vaccine and therefore, this dog was excluded because the obtained antibody titer could be vaccine-induced. One dog occurred twice in the dataset because the antibody titer was determined twice that year, therefore only the first antibody titer was included in the study. Of two dogs, the medical files were incomplete. One dog travelled to Austria and Germany, two dogs travelled to Romania and no history of travelling to an endemic area could be found in the medical records of three other dogs. In total, these ten dogs were excluded from the dataset. Therefore, the medical records of 118 dogs fulfilled the selection criteria and were studied retrospectively (figure 1). Table 2 shows the signalment of the dogs included in this study. The dogs were either born in or travelled to an endemic area. Countries they were born in, were Spain (n=42), Greece (n=32), Portugal (n=16), Italy (n=4), Turkey (n=2), France (n=1), Bulgaria (n=1), Cyprus (n=1) and Egypt (n=1). Other dogs were born in The Netherlands, but travelled to Spain, France and/or Italy.

In this dataset, 38 dogs had a positive (>1:40) titer and 80 dogs had a negative (≤1:40) titer. Table 3 shows the distribution of the results of the DAT antibody titers. In the positive group, 18 dogs had received or were receiving treatment for their Leishmaniosis when the antibody titer was performed due to positive results in a previously performed test.

Table 2 Dog signalment, n = 118

	Negative dogs (n=80)	Positive dogs (n=38)
Age (years)		
mean	4	5
range	0,4-14,1	0,5-12,8
Gender (% neutered)		
females	44 (84%)	24 (79%)
males	36 (75%)	14 (43%)
Breed		
cross breed	52	23
other breed	28	15

Table 3 Distribution of DAT titer results, n=118

Height of the DAT titer	Number of dogs
≤1:40	80
1:640	2
1:1280	2
1:2560	3
1:5120	4
1:10240	5
1:20480	14
1:40960	6
1:81920	2

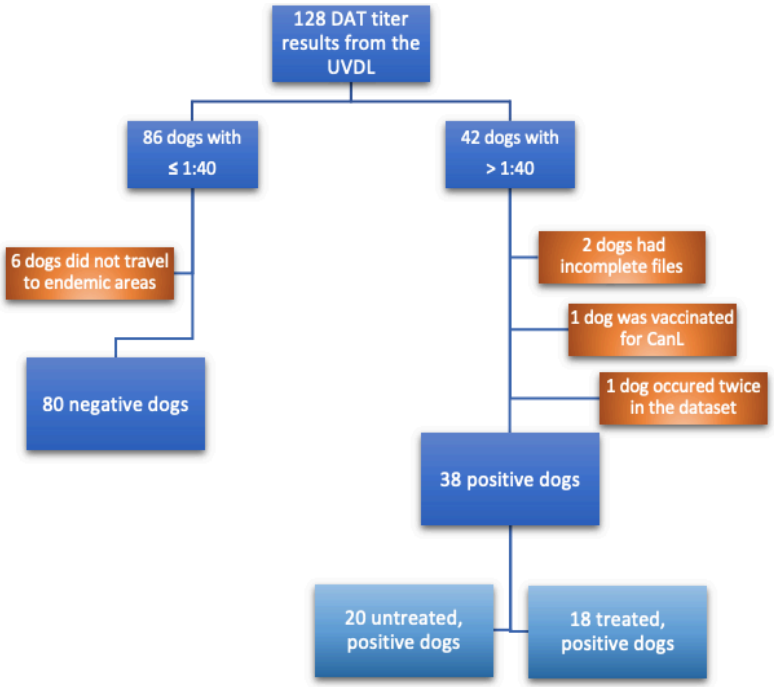

Figure 1 Flowchart representing the case selection process used in this study

3.2. Clinical signs

Frequency of clinical signs of dogs with negative and positive DAT titers that was scored in the files is shown in Table 4. In total, eleven clinical signs were shown significantly more frequently by the positive dogs then the negative dogs. The most commonly shown clinical signs were lymphadenopathy (52,6%, OR=3,6) alopecia (42,1%, OR=10,9) and crustae on the ears (36,8%, OR=7,2). Other significant symptoms were decreased endurance, weight loss, diarrhea, squamae, ulcerative skin lesions, hyperkeratosis and onychopathy. Most interestingly, diarrhea had the highest Odds-ratio of 28,2. Some clinical signs were shown by a few of the positive dogs, but not by any of the negative dogs such as muscle atrophy, onychopathy, depigmentation, dry exfoliative dermatitis, myositis and conjunctivitis. For all clinical symptoms, percentages were higher in the positive group then in the negative group, resulting in no OR between zero and one.

Table 4 Frequency of clinical signs of dogs with negative and positive DAT titers, the Odds ratio and P-value, n = 118

Clinical signs	Number of negative dogs (n=80)	%	Number of positive dogs (n=38)	%	OR
Lymphadenopathy	19	23,5%	20	52,6%	**3,6*****
Decreased endurance	8	9,9%	13	34,2%	**4,7*****
Weight loss	3	3,7%	11	28,9%	**10,5*****
Diarrhea	1	1,2%	10	26,3%	**28,2*****
Decreased appetite	7	8,6%	8	21,1%	2.8 ^{NS}
Lameness	3	3,7%	6	15,8%	**4,8***
Vomiting	4	4,9%	6	15,8%	3,6^{NS}
Polyuria/ polydipsia	5	7,4%	6	15,8%	2,8^{NS}
Fever	3	3,7%	3	7,9%	2,2^{NS}
Muscle atrophy	0	0,0%	1	2,6%	6,4^{NS}
Alopecia	5	6,2%	16	42,1%	**10,9*****
Crustae on ears	6	7,4%	14	36,8%	**7,2*****
Erythema	14	17,3%	12	31,6%	2,2^{NS}
Squamae	5	6,2%	11	28,9%	**6,1*****
Crustae on body	11	13,6%	9	23,7%	1,9^{NS}
Ulcerative lesions skin	1	1,2%	5	13,2%	**12,0***
Pale mucous membranes	5	6,2%	5	13,2%	2,3^{NS}
Hyperkeratosis	1	1,2%	4	10,5%	**9,3***
Papular/nodular dermatitis	1	1,2%	3	7,9%	6,8^{NS}
Onychopathy	0	0,0%	3	7,9%	**15,9***
Pustules	3	3,7%	3	7,9%	2,2^{NS}
Hyperpigmentation	1	1,2%	3	7,9%	6,8^{NS}
Depigmentation	0	0,0%	2	5,3%	11,0^{NS}
Dry exfoliative dermatitis	0	0,0%	1	2,6%	6,4^{NS}
Mucocutaneous ulcerative lesions	1	1,2%	1	2,6%	2,1^{NS}
Ulcerations footpads	0	0,0%	0	0,0%	ND
Epistaxis	1	1,2%	3	7,9%	6,8^{NS}
Myositis	0	0,0%	1	2,6%	6,4^{NS}
Conjunctivitis	0	0,0%	1	2,6%	6,4^{NS}
Splenomegaly	1	1,2%	1	2,6%	2,1^{NS}
Uveitis	0	0,0%	0	0,0%	ND

OR = Odds ratio, ***= $P < .001$, **= $P < .01$, *= $P < .05$, NS= not significant, ND= not determined, because these symptoms were not shown by either the positive and the negative dogs.

3.3. Hematological and biochemical manifestations

Hematologic and biochemical manifestations of positive, untreated dogs and negative dogs are represented in Table 5. Part of the data was normally distributed, being hematocrit, lymphocytes, leucocytes, beta-2 globulins, total proteins, albumin, urea and creatinine. The rest of the data was not normally distributed. When hematologic and biochemistry results were compared between these two groups, statistically significant differences were found for gamma-globulins (P = .037), total protein (P = .002) and UPCR (P = .022). No significant differences were found for the other biochemical and hematologic parameters as can be seen in Table 5.

Table 5 The mean, standard deviation, P-value and laboratory reference of the hematological and biochemical parameters of negative and positive, untreated dogs, n = 100

Parameter (units)	Number of negative dogs	Number of positive, untreated dogs	Negative mean (± SD)	Positive mean (± SD)	*P*	Laboratory reference
Hematology						
Hematocrit (L/L)	75	20	0,45 (±0,07)	0,44 (±0,09)	0,319	0,42 - 0,61
Reticulocytes (x10^9/L)	67	13	40,45 (±77,4)	40,34 (±26,41)	0,072	5,2 - 126,5
Lymphocytes (10^9/L)	75	20	2,74 (±1,71)	2,3 (±1,06)	0,185	0,8 - 4,7
Leukocytes (10^9/L)	74	20	10,48 (±3,66)	9,37 (±3,93)	0,267	4,5 - 14,6
Neutrophils (10^9/L)	75	20	6,9 (±2,78)	6,04 (±3,53)	0,097	2,9 - 11,0
Monocytes (10^9/L)	75	20	0,49 (±0,26)	0,52 (±0,32)	0,987	0,0 - 0,9
Eosinophils (10^9/L)	75	20	0,58 (±0,6)	0,58 (±0,5)	0,864	0,0 - 1,6
Thrombocytes (10^9/L)	74	18	246,45 (±76,42)	230,72 (±118,33)	0,191	144 - 603
Biochemistry						
Alpha-1 globulins (g/L)	11	16	2,5 (±0,52)	3 (±0,73)	0,142	5 - 10
Alpha-2 globulins (g/L)	11	16	9,5 (±3,17)	9,06 (±1,48)	0,930	4 - 10
Beta-1 globulins (g/L)	11	16	4 (±2,49)	3,25 (±0,77)	0,979	3 - 10
Beta-2 globulins (g/L)	11	16	10,7 (±2,76)	10,81 (±2,54)	0,951	4 - 10
Gamma globulins (g/L)	11	16	**9,5 (±8,27)**	**20,38 (±16,45)**	**0,037**	3 - 9
Total protein (g/L)	22	17	**60,5 (±9,31)**	**73,59 (±13,55)**	**0,002**	55 - 72
Albumin (g/L)	22	17	26,8 (±7,63)	28,29 (±6,74)	0,721	26 - 37
Urea (mmol/L)	15	9	9,1 (±8,48)	5,86 (±1,86)	0,411	3,0 - 12,5
Creatinine (umol/L)	17	16	89,1 (±59,6)	58,25 (±19)	0,085	50 - 129
UPCR (no unit)	10	13	**2 (±2,92)**	**0,16 (±0,09)**	**0,022**	<0,01

UPCR urinary protein to creatinine ratio

The most frequent shown hematologic and biochemical manifestations are listed in Table 6. Anemia was shown 25% of the positive dogs of which all were non-regenerative whereas 13,8% of the negative dogs showed anemia of which 81,8% was non-regenerative. Thrombocytopenia was not a frequently shown manifestation in the blood results: 20% of the positive dogs and 5% of the negative dogs showed this finding. Of the positive dogs, 35% showed a hyperproteinemia whereas only 2,5% of the negative dogs showed this alteration. Hypoalbuminemia was found in positive and negative dogs (30% and 8,8% respectively). More than half of the positive dogs had a hypergammaglobulinemia (55%) whereas only 3,8% of the negative dogs showed this alteration. A normal leucogram was the most common finding in both positive and negative dogs. Of the positive dogs, 10% showed leucopenia and 5% showed leukocytosis because of an increase in neutrophil concentration.

Table 6 Most common hematological and biochemical manifestations of negative and positive, untreated dogs, n = 100. See Appendix 2 for definitions.

hematological and biochemical manifestations	Number of negative dogs (%) n = 80	number of positive, untreated dogs (%) n = 20
Anemia	11 (13,8%)	5 (25%)
Non-regenerative	*81,8%*	*100%*
Thrombocytopenia	4 (5%)	4 (20%)
Hyperproteinemia	2 (2,5%)	7 (35%)
Hypoalbuminemia	7 (8,8%)	6 (30%)
Hypergammaglobulinemia	3 (3,8%)	11 (55%)
Leukocytosis	10 (12,5%)	1 (5%)
Leukopenia	3 (3,8%)	2 (10%)
Increased UPCR (>0.5)	4 (5%)	0 (0%)

3.4. Correlation between blood parameters and height of the DAT titer

The degree of association between the blood parameters and the DAT titer of positive, untreated dogs are reported in Table 7. The correlations between the blood parameters and the height of the DAT titer were not significant, with the exception of leukocytes, r_s = -.56, $P < .01$ (two-tailed) and urea, r_s = .70, $P < .05$ (two-tailed). The correlation plots are shown in Figure 2.

Table 7 Rho and P-values of the correlation between DAT titers of positive, untreated dogs and laboratory parameters.

Parameter (units)	Number of values	Spearman (r_s)
Hematology		
Hematocrit (L/L)	20	.15
Reticulocytes (x10^9/L)	13	.25
Lymphocytes (10^9/L)	20	-.27
Leukocytes (10^9/L)	**20**	**-.56****
Neutrophils (10^9/L)	20	-.43
Monocytes (10^9/L)	20	-.26
Eosinophils (10^9/L)	20	-.33
Thrombocytes (10^9/L)	18	-.19
Biochemistry		
Alpha-1 globulins (g/L)	16	.15
Alpha-2 globulins (g/L)	16	-.10
Beta-1 globulins (g/L)	16	.29
Beta-2 globulins (g/L)	16	.04
Gamma globulins (g/L)	16	.23
Total protein (g/L)	17	.30
Albumin (g/L)	17	-.20
Urea (mmol/L)	**9**	**.70***
Creatinine (umol/L)	16	.02
UPCR (no unit)	13	.18

UPCR urinary protein to creatinine ratio **= $P < .01$, *= $P < .05$

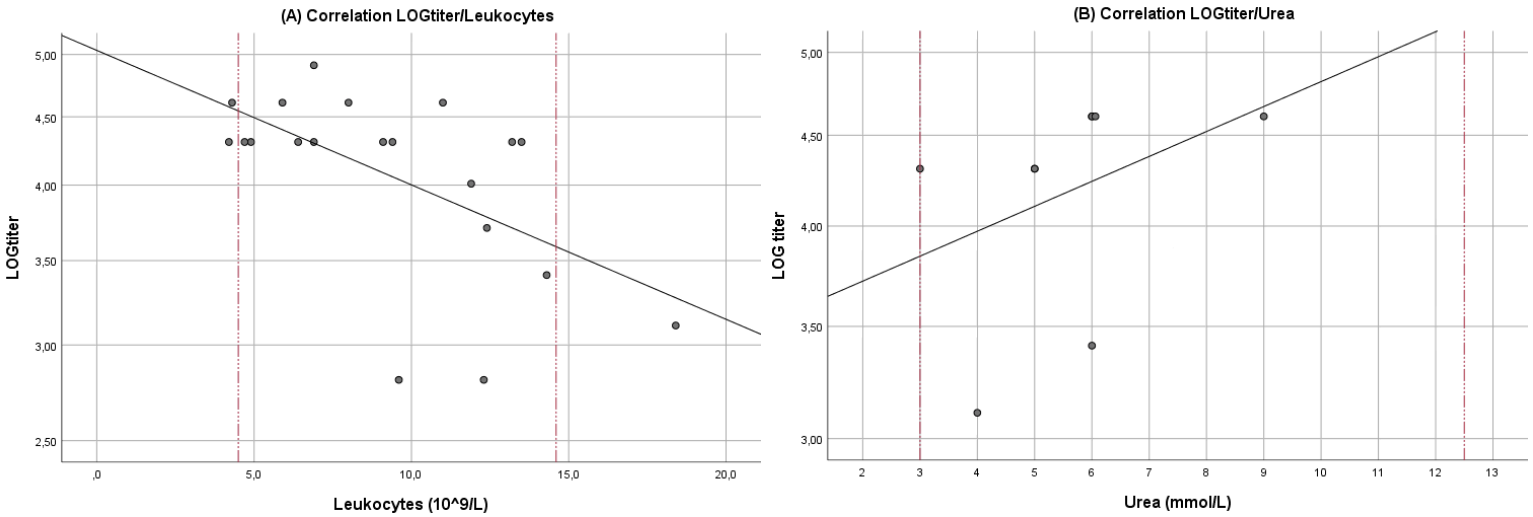

Figure 2 Correlation between the height of the DAT titer and the level of leucocytes (A) and urea (B). The red, interrupted, vertical lines represent the upper and lower laboratory reference values.

3.5. Correlation between staging systems and height of the DAT titer

All 20 positive, untreated dogs were staged in the most appropriate category of the three classification systems. None of the data was normally distributed according to the Shapiro-Wilk test. The results of the assignment of dogs into the different categories of the clinical staging systems are shown in Table 8. Figure 3 shows the boxplots of the height of the DAT titers in each classification system and their stages. Only in the Leishvet staging system, some dogs were staged as "unclassified" (n=3). No significant level of association between the DAT score and the staging systems was found as can be seen in Table 8.

Table 8 Distribution of the positive, untreated dogs in the three classification systems and the Rho and P-value of the correlation between the DAT titer and classification systems, n = 20

System	Stage	Number of dogs	Spearman (r_s)
Amusategui et al. (2003)	Unclassified	0	$0,21^{NS}$
	Initial	5	
	Established	14	
	Advanced	1	
Leishvet - Solano-Gallego et al. (2009)	Unclassified	3	$0,06^{NS}$
	Mild	6	
	Moderate	9	
	Severe	2	
	Very severe	0	
CLWG – Paltrinieri et al. (2010)	Unclassified	0	$0,21^{NS}$
	Exposed	0	
	Infected	5	
	Sick	14	
	Severely sick	1	
	Sick unresponsive/ relapse	0	

NS = Not significant

4. Discussion

Key findings

We found that positive dogs showed several symptoms (lymphadenopathy, alopecia, crustae on the ears, decreased endurance, weight loss, diarrhea, lameness, squamae, ulcerative lesions on the skin, hyperkeratosis and onychopathy) significantly more frequently than negative dogs. Also, we found that positive dogs had significant higher level of Gamma-globulins, total protein but a lower UPCR. A significant positive correlation between urea and the height of the antibody titer and a significant negative correlation between leucocytes and the height of the antibody titer was found. Moreover, no correlation was found between the height of the antibody titer and the three different clinical staging systems.

Clinical symptoms

The most frequent clinical findings in positive dogs were lymphadenopathy (52,6%, OR=3,6) alopecia (42,1%, OR=10,9) and crustae on the ears (36,8%, OR=7,2). But also decreased endurance (OR=4,7), weight loss (OR=10,5), diarrhea (OR=28,2), lameness (OR=4,8), squamae (OR=6,1), ulcerative lesions on the skin (OR=12,0), hyperkeratosis (OR=9,3) and onychopathy (OR=15,9) were shown significantly more frequent by positive dogs then by negative dogs. These symptoms are frequently found in dogs with CanL.[8,12,15,22–25] Some of these symptoms are more general, unspecific whereas others are more specific for CanL. Considering that almost halve of the positive dogs did receive treatment for more than 4 weeks, some of the clinical signs of these treated dogs might have been suppressed.

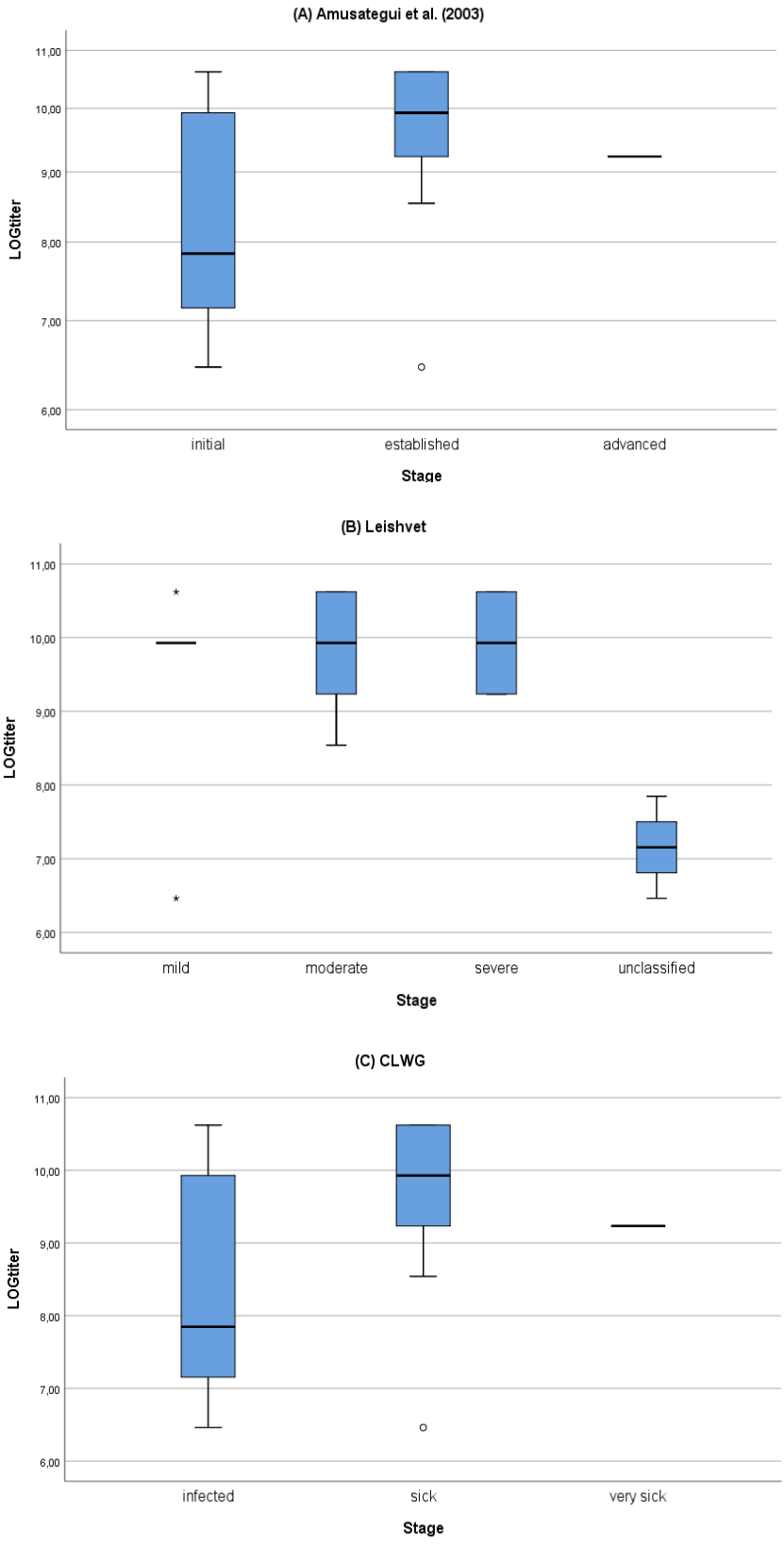

Figure 3 Boxplots of the LOG of the DAT titers in each classification system (A) Amusategui et al. 2003 (B) Leishvet and (C) CLWG. The black line in the middle of the boxes represents the mean of the stage and dots represent the outliers, n = 20

Most studies also found a frequency of lymphadenopathy to be above 50%, except for Pereira et al. (2020) who only found that 21% of patients showed this symptom.[15] In the current study, splenomegaly and ulcerations on the footpads were never shown by positive dogs. Other studies also show a very low frequency of cases showing splenomegaly and ulcerations on the footpads.[12,15,18,22] However, Ciaramella et al. (1997) found that 50% of infected dogs showed splenomegaly.[26] Splenomegaly is caused by immune cells that proliferate and infiltrate spleen this results in hyperplasia.[25] The variety in frequencies of splenomegaly found, could be because the enlargement is usually not severe enough to be palpated on physical examination and is mostly detected by ultrasound examination. [22] An ultrasound was not performed on the positive dogs in this study.

Regarding the absence of cases displaying uveitis, one other study also concluded zero cases of this symptom.[27] Even though, this is a symptom that can be seen frequently in dogs with CanL, concurrent with other ophthalmological pathologies.[13,22,23,26,28,29] Anterior uveitis is one of the most common ophthalmological symptom found and ocular lesions can even be the only symptom shown by dogs infected with CanL without any identifiable systemic signs.[28]

Most interestingly, the odds of a positive dog showing diarrhea were 28 times higher than the odds of a negative dogs showing this problem. In total 26,3% of the positive dogs, showed diarrhea. Other research concludes a wide variation of occurrence of this symptom, but mostly lower then found in the current study.[13,17,23] A possible explanation why diarrhea is shown by dogs with a Leishmania infection is because infected dogs show a high parasite load in the whole length of the gastrointestinal tract, especially in the colon and caecum.[30] On histological evaluation, an increased number of plasma cells, lymphocytes and macrophages were found in infected dogs. The macrophages contained many Leishmania amastigotes and were seen in both the mucosal, muscular and submucosal layer of the intestinal wall, despite the clinical status of the dogs. This parasite burden in the intestine could lead to a chronic inflammatory process in the crypts of Lieberkühn with degeneration and cellular swelling. This causes small erosions in the mucosal surface, limiting the surface of the large bowel available for absorption and thus causing diarrhea.[31] Another study showed that 32,3% of infected dogs presented with colitis and on endoscopic examination, the mucosa of the colon showed hyperemia, edema and erosions.[32] However, other causes of diarrhea, like idiopathic large bowel inflammatory diseases, food induced diarrhea or other infections [25], have to be taken in account as these other causes were not excluded in this study. Therefore, it is unknown in how many of the cases in this study CanL colitis is the main explanation for the occurring diarrhea since it was beyond the scope of this study.

Hematological and biochemical manifestations

When looking at the blood results of positive, untreated dogs, a normal leucogram was the most common finding. Together with the absence of a relationship between the height of the titer and the counts of total lymphocytes, neutrophils, monocytes and eosinophils showed that the infection has little influence on these parameters. But leucocyte counts do appear to be influenced by the disease, as the count of lymphocytes decreased with the height of the titer (r_s =-.56, P <.01). This may be attributed to the immunosuppressive nature of CanL as concluded by two studies who found lower leucocytes levels in symptomatic dogs.[19,33] Still, in the current study, almost all leucocyte counts were within reference rate which was also most frequently determined in other research. [12,14,15,17,34]

This study found a lower percentage of positive dogs with non-regenerative anemia then other literature describes. [8,12,22,26] Anemia due to CanL is multifactorial and is related to chronic inflammatory disease, hemorrhage, chronic renal failure, hemolysis, bone marrow medullar hypoplasia or aplasia.[22,35] However, the mean of the hematocrit of the positive, untreated dogs was within reference rate which indicates that the dogs could be recently infected and are not yet chronically diseased.

This analysis revealed hypergammaglobulinemia, hyperproteinemia and hypoalbuminemia as the main laboratory findings in dogs infected with Leishmaniasis. When looking at the group of untreated positive dogs, the mean of gamma-globulins and total protein were statistically higher than those of the negative group. According to the literature, these are considered common findings in dogs infected with CanL. [9,14,17,22,24,26,36] The increase of gamma-globulins is possibly due to polyclonal activation of B-lymphocytes, triggered by Leishmania antigen, the synthesis of non-specific antibodies and circulating immunocomplexes.[8,9] However, the percentages found in this current study are lower than those found in other studies. Mélendez-lazo et al. (2018) found 72,5% of dogs showed hypergammaglobulinemia and 54,9% showed hypoalbuminemia.[12] Whereas this current study found respectively 55% and 30%. Proverbio et al. (2016) found even higher percentages of hypergammaglobulinemia and hypoalbuminemia (respectively 100% and 95%). [37]

A higher mean of the UPCR was found in negative dogs then in positive, untreated dogs. This is contradictory to what other studies concluded. Normally, proteinuria is a frequent finding in dogs with CanL.[7] This study found that none of the untreated, positive dogs had an increase in the UPCR. Other studies found high percentages of their studied positive dogs show proteinuria, even as high as 84,7 percent.[38] Most often, a proteinuria without azotemia can be found in dogs with CanL but the presence of histological lesions in 100% of the evaluated dogs has also been described.[39,40] Even though there is a high prevalence of renal abnormalities, azotemia is still a uncommon finding as creatinine is not sensitive enough to detect early stages of renal failure.[41] Creatinine levels in the blood will start to rise when the majority of nephrons are dysfunctional due to glomerulonephritis and/or tubulointerstitial nephritis. The first is associated with circulating immune complexes being deposited in the glomeruli, inducing inflammatory changes.[7,39] These pathologies are chronic processes and are shown rather late in the progression of the disease. This could be one of the reasons why none of the positive, untreated dogs had proteinuria in this study as they were diagnosed early in their disease. In this study, three negative dogs had a very high UPCR. Two of these dogs were diagnosed with chronic kidney disease and one was tested positive for Ehrlichia which could have contributed to the renal failure. Nevertheless, it is important to assess renal function in dogs diagnosed with CanL because this has a major impact on the therapeutic decisions and prognosis.[7]

According to the present work, a significant positive correlation between urea and the height of the titer was found (r_s=.70, P <.05) though no significant difference was found between the urea levels of untreated positive and negative dogs. All urea levels of the positive untreated dogs were within reference interval. This is contradictory to other studies, who did find an elevation of the urea level of positive dogs.[14,16,42] Also, no correlation between the height of the antibody titer and the urea levels in the blood has been found by Melendez-lazo et al. (2018).[12] A significant negative correlation was found between the number of leucocytes and the height of the titer. Again, almost all leucocyte counts of the positive untreated dogs were within reference intervals. This correlation has not been found in other studies who did find a negative correlation between, for example, hematocrit and the antibody levels.[43] Also, a correlation between antibody level and total protein and gamma-globulins has been reported. [17,20,43] This study found no such correlations, possibly due to the small cohort used (n=38).

Staging systems

This research found no correlation between the height of the antibody level and the three staging systems. Only one other study assessed this correlation between their own clinical scoring system and the height of the antibody titer.[20] They concluded a positive correlation but more research would be desirable as the clinical scoring system is not widely used or validated. Amusategui et al. (2003) researched their own staging system to determine the relation between IFAT titers and the severity of the clinical signs but found no relationship.[17] However, when comparing the IFAT titer within the different stages, they found that dogs in the initial stage showed the lowest antibody

titers. No studies were found that assessed the correlation between the height of the antibody titer and the Leishvet and CLWG staging systems.

Problems were seen when classifying dogs into the categories of the clinical staging systems established by several authors because of the different criteria used in each one. Besides, it is important to keep in mind that all dogs were classified retrospectively by one person. This gives the possibility of an observer bias in this cohort. Also, when clinical signs are used for assignment to the different categories, subjective observations should be avoided. Moreover, laboratory results should not be overlapped between categories because this causes difficulties when assigning each dog to a category.[12]

The Leishvet staging systems was the only one for which dogs could not be classified. The main reason being the definition of their stages. The first stage (mild) requires the dog to show lymphadenomegaly and papular dermatitis whereas their second stage (moderate) requires the dog to show skin problems, anorexia, weight loss, fever or epistaxis but most also show the lymphadenomegaly and papular dermatitis from the previous stage. Therefore, when dogs did not show lymphadenomegaly or papular dermatitis, they could not be classified in any of these two stages. This was the case for three positive dogs who showed other symptoms like lameness, gastrointestinal problems or were asymptomatic.

Strengths and limitations

One limitation of this study is the fact that the group of dogs with negative titers could not truly be a representative control group to compare to the positive dogs. Part of these dogs were healthy, asymptomatic dogs but the other part of this group were sick dogs of which the anamnesis and clinical signs in the file were suggestive for CanL after which the antibody level was assessed. This leads to a non-homogeneous group of negative dogs and could lead to a selection bias as the dogs are not randomly selected. This makes it harder to draw conclusions on identifying the most indicative clinical signs of dogs with CanL. But this selection bias on the group with negative dogs would not have an influence on the results of the correlations. Besides, not every file was as complete or detailed as others and many different people working at the UUCCA assessed the dogs upon their visit which could lead to another observer bias.

Another limitation of this study is the small dataset, especially the number of untreated, positive dogs. Therefore, it is difficult to assess significant differences between this group and the negative dogs and to determine correlations between the blood parameters and the height of the antibody titer. It is necessary to only use dogs that have not received any treatment for their Leishmaniasis because treatment has an effect on both antibody level and blood parameters. This can be seen as quickly as within thirty days of treatment.[43] Dogs treated with Allopurinol and meglumine antimoniate showed a significant decrease in level of antibodies and blood manifestations after one month. Although another study found no decrease in antibodies, they did find clinical, hematological and biochemical improvements after thirty days of treatment.[44] However, the dogs included in their study were treated with different medication then Allopurinol. Overall, titers tend to decrease in the majority of treated dogs, regardless the therapeutic protocol. Some dogs can even turn seronegative after four months of treatment.[33,43] Therefore, animals that were treated for CanL could not be included in this study when tests for correlation and differences in mean blood values were performed. Another reason why no correlations with the antibody titer was found, could be because the dataset lacked dogs with positive antibody titers in the lower range. Most dogs were positive with an antibody titer of 1:20480 or higher. Therefore, it could be more difficult to assess correlations. However, this could likely be more representable of the usual status of patients when they are diagnosed with CanL.

Further studies are needed in order to explore the possible correlation between the height of the antibody level and clinical and clinicopathological manifestations. These studies should include a larger dataset with positive untreated animals with a wider distribution in antibody levels.

5. Conclusion

In conclusion, the results show that lymphadenomegaly, alopecia and crustae on the ears were the most common clinical signs found in dogs with CanL. The most frequent hematological and biochemical findings in these dogs, were a hypergammaglobulinemia, hypoalbuminemia and hyperproteinemia. Positive, untreated dogs showed significant higher level of gamma globulins ($P < .05$), higher total protein ($P < .001$) but controversially to many studies, they showed a lower UPCR ($P < .05$). A significant positive correlation between the height of the antibody level and the level of urea ($r_s = .70$, $P < .05$) and a negative correlation between the height of the antibody level and the level of leucocytes ($r_s = -0.56$, $P < .01$) was found. No correlation was found between the height of the antibody level and three different clinical staging systems.

The results of this study partly support the idea that DAT titers are related to the hematological and biochemical manifestations but reject the hypothesis that the DAT titer is correlated to clinical manifestations. However, it does reinforce the importance of clinical hematological and biochemical parameters for diagnosis and therapeutic decisions and evaluation.

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Appendix 1. Informed Consent for dog owners

Universitair Diergeneeskundig Centrum Utrecht
Universiteitskliniek voor Gezelschapsdieren

Informatiebrief en toestemmingsformulier

Onderzoek naar de klinische verschijnselen van Leishmania

Afdeling Hematologie, Universiteitskliniek Gezelschapsdieren, Universiteit Utrecht
Dr. C. J. Piek, specialist interne ziekten gezelschapsdieren
Drs. M.V. Voorhorst, specialist interne ziekten gezelschapsdieren

Graag vragen wij u middels dit schrijven toestemming voor het beoordelen van het medische dossier van uw hond en voor de mogelijkheid om u eventueel aanvullende vragen te stellen ten behoeve van een onderzoek van het departement Geneeskunde van Gezelschapsdieren van de faculteit Diergeneeskunde van de Universiteit Utrecht. Het is voor deelname aan een wetenschappelijk onderzoek vereist, dat u een schriftelijke verklaring geeft dat u volledig bent ingelicht over het onderzoek en dat u bereid bent om mee te werken. Dit wordt 'informed consent'; ofwel geïnformeerde toestemming genoemd. U zult door middel van dit document uitgelegd krijgen wat de opzet is van het onderzoek, wat uw medewerking precies zal inhouden, en wat de voordelen, nadelen en mogelijke risico's zijn. Ook zal u worden uitgelegd hoe er met de resultaten van het onderzoek wordt omgegaan zodat uw privacy gewaarborgd is.

Het doel van dit project
Door middel van dit onderzoek willen wij uitzoeken of er een relatie is tussen de hoeveelheid antilichamen in het bloed en de klinische verschijnselen van een hond met Leishmania. Het is het uiteindelijke doel om een zo goed mogelijke prognose en behandeling te kunnen formuleren voor deze patiënten gebaseerd op gedegen onderzoeks-bewijs.

Algemene informatie
Uw hond is afkomstig uit Zuid-Europa of heeft er een deel van zijn leven doorgebracht. Een van de hondenziektes die in Zuid-Europa veel voorkomt is Leishmaniasis. Dit is een ziekte die door een zandvlieg wordt overgebracht. Ziektes, waarbij een insect zorgt voor besmetting, zijn vector-overdraagbare aandoeningen. Omdat de zandvlieg die Leishmania overdraagt in Nederland niet voorkomt kunnen honden in Nederland de ziekte niet oplopen. Een hond die in Zuid-Europa geïnfecteerd geraakt is heeft daar in het algemeen een zomer doorgebracht. Onderzoek naar besmetting en ontwikkeling van de ziekte is van belang om tijdig met medicatie in het ziekteproces te kunnen ingrijpen.

Waarom is mijn hond hiervoor geselecteerd?
Uw hond is voor dit onderzoek geselecteerd omdat bij uw hond Leishmania is gediagnosticeerd of omdat hij op dit moment sterk verdacht wordt van deze ziekte.

Wat zouden we graag van u / uw hond willen weten?
Voor dit onderzoek zouden we graag inzage hebben in het medische dossier van uw hond. We zijn geïnteresseerd in de uitslagen van het lichamelijk onderzoek en bloedonderzoek bij uw hond. Indien er na het bestuderen van het medisch dossier toch nog vragen zijn, zouden we u eventueel aanvullend kunnen benaderen.

Wie voert het onderzoek uit?
Het onderzoek staat onder leiding van dr. C.J. Piek, Internist voor Gezelschapsdieren, hoofd van de afdeling Hematologie en Vector-overgedragen Infectieziektes en drs. M.J. Voorhorst, Internist voor gezelschapsdieren en tevens medewerker van de afdeling Hematologie en Vector-overgedragen Infectieziektes. Studenten van de laatste fase van de opleiding Diergeneeskunde hebben een ondersteunende functie, als onderdeel van de studie.

Wat zijn negatieve/positieve gevolgen van meewerken aan dit onderzoek?

Wij vragen aan u inzage in het medisch dossier van uw hond wat door uw eigen dierenarts is bijgehouden. U hoeft hiervoor niet langs te komen. Uw hond hoeft geen aanvullende onderzoeken te ondergaan. Er zijn voor u geen kosten verbonden aan dit onderzoek. Wij hopen mede op basis van uw gegevens uiteindelijk zo goed mogelijke richtlijnen te kunnen uitgeven voor de beste behandeling voor honden met deze aandoening. Wij streven ernaar dat (toekomstige) honden met deze aandoening hierdoor een betere behandeling en een beter en langer leven kunnen hebben.

Wat gebeurt er met de gegevens/informatie?
De gegevens die tijdens dit project zijn verzameld worden gebruikt voor een publicatie in een wetenschappelijk tijdschrift. De gegevens zullen niet te herleiden zijn tot het individuele dier.

Vertrouwelijk- wie heeft er toegang tot de data?
Persoonsgegevens die worden verzameld tijdens deze studie worden vertrouwelijk behandeld. Persoonsgegevens zullen nooit worden vermeld in publicaties of presentaties. In publicaties of presentaties naar aanleiding van het onderzoek worden, naast de uitkomsten van het onderzoek, uitsluitend de uiterlijke kenmerken van uw hond, zoals ras, geslacht, leeftijd en de relevante delen van de ziektegeschiedenis en medische gegevens anoniem gepubliceerd. De onderzoekers hebben wel toegang tot de persoonlijke gegevens, zodat er wel correspondentie kan plaatsvinden. De gegevens worden bewaard gedurende het onderzoek, indien u toestemming geeft wordt het bewaard voor vervolgonderzoek.

Kan ik mij terugtrekken uit dit onderzoek?
U kunt zich terugtrekken uit dit onderzoek op elk moment zonder daarvoor een reden te geven. Dit kunt u doen door een email te sturen naar: leishmania-onderzoek@uu.nl

Wie kunt u benaderen bij vragen over dit onderzoek?
Email: Leishmania-onderzoek@uu.nl

Op de volgende pagina vindt u het toestemmingsformulier. Hierop kunt u aangeven of, en onder welke voorwaarde u toestemming geeft voor deelname aan het onderzoek. Wij vragen u dit formulier ingevuld en ondertekend naar ons te mailen.
Bij voorbaat hartelijk dank.

Toestemmingsverklaring

Ik bevestig dat ik het informatieformulier heb gelezen. Ik begrijp de informatie. Ik heb voldoende tijd gehad om over deelname na te denken.
Ik weet dat mijn deelname geheel vrijwillig is en dat ik mijn toestemming op ieder moment kan intrekken zonder dat ik daarvoor een reden moet geven.
Hieronder geef ik mijn keuze aan:

☐ Ik geef toestemming voor het toesturen van en inzage in het medisch dossier van mijn hond door mijn dierenarts zoals in de informatiebrief is beschreven. Ik geef toestemming om de gegevens te verwerken voor de doeleinden zoals beschreven in de informatiebrief.

☐ Ik wil toestemming geven voor het toesturen van en inzage in het medisch dossier van mijn hond door mijn dierenarts zoals in de informatiebrief is beschreven, maar ik heb nog aanvullende vragen aangaande het project. Ik geef toestemming om de gegevens te verwerken voor de doeleinden zoals beschreven in de informatiebrief. We nemen contact met u op om uw vragen te beantwoorden.

☐ Ik geef geen toestemming voor het toesturen van en inzage in het medisch dossier van mijn hond door mijn dierenarts zoals in de informatiebrief is beschreven.

☐ Ik geef geen toestemming om benaderd te worden voor aanvullende vragen met betrekking tot het onderzoek.

Naam eigenaar: _____

Datum: _____

Handtekening: _____

Persoonlijke gegevens

Adres: _____

Postcode: _____

Emailadres: _____

Telefoonnummer: _____

Naam hond: _____

Chipnummer: _____

Ras: _____

Geboortedatum: _____

In Nederland sinds: _____

Appendix 2. List of definitions and abbreviations

CanL = Canine Leishmaniosis
DAT = Direct Agglutination Test
UVDL = University Utrecht Veterinary Diagnostic Laboratory
UUCCA = University of Utrecht Clinic for Companion Animals
UPCR = Urinary Protein to Creatinine Ratio

Anemia = Hematocrit levels below 0,42 L/L
Thrombocytopenia = Platelet levels below 144 $x10^9$/L
Hypoalbuminemia = Albumin levels below 26 g/L
Hypergammaglobulinemia = Gamma-globulin levels above 9 g/L
Leukocytosis = Leucocyte count above 14,6 $x10^9$/L
Leukopenia = Leucocyte count below 4,5 $x10^9$/L