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ORIGINAL ARTICLE

Hematological, biochemical, serological, and molecular monitoring of blood donor dogs vaccinated with CaniLeish® for the prevention of Leishmaniosis

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Abstract

The use of systemic preventive measures in dogs living in areas with Leishmania infection is advisable, particularly in blood donors. The aim of this study was to monitor healthy blood-donor dogs immunized with the vaccine CaniLeish® by means of hematological, biochemical (serum total protein, albumin, protein electrophoresis, and C-reactive protein), serological (Speed Leish K™ and IFAT), and molecular assays (qualitative PCR, qlPCR). Twenty-four dogs were monitored at (T0), second (T1), third (T2) immunizations, and 2 months (T3) after the initial immunization and at the first (T4), second (T5), and third (T6) annual booster. The results were as follows: slight hyperproteinemia (29.5%, $p > 0.05$) observed throughout the monitoring period (T0-T6); increase in C-reactive protein (46.4%, $p < 0.05$) (at T3-T6); significant alterations in β-1 and β-2 globulin fractions (as absolute and percentage values, $p < 0.05$), Speed Leish K™ assay negative at T0 and T4-T6, positive IFAT titres of 1/40 (9.5%) at T0, and T3-T6, 1/80 (8.6%) at T1-T4, 1/160 (6.7%) at T1-T2 and T4, and 1/320 (1.9%) at T2 and T4; and qlPCR positive in 7.7% of samples at T3-T6. The vaccination with Canileish® induced changes in a few clinico-pathological and serological markers, which are likely associated with the activation of the immune response. These changes should be carefully considered when evaluating vaccinated dogs included in blood transfusion programs. The use of the CaniLeish® vaccine could be recommended as an additional preventive measure and could represent an important practice in the field of canine transfusion medicine.

Keywords CaniLeish® · Blood donor-dogs · PCR · Serology · Leishmaniosis · Vector-borne diseases

Introduction

Canine Leishmaniosis (CanL) is a zoonotic disease caused by the protozoan parasite *Leishmania infantum* and represents a serious public health problem in many parts of the world, due to its zoonotic nature. The domestic dog is considered to be the main reservoir of human infection, and phlebotomine sand flies are the biological vectors of the parasite (EFSA AHAW 2015).

An important epidemiological feature of CanL in endemic areas is a high prevalence of infection despite a lower prevalence of clinical disease (Pennisi 2014).

A few studies have shown that *Leishmania* can be transmitted both by whole blood/mononuclear fractions and by plasma transfusion, irrespective of the clinical condition (Tabar et al. 2008; Owens et al. 2001 and De Freitas et al. 2006).

The recently revised Italian guidelines on veterinary transfusion medicine established that blood donor dogs should be healthy animals. The guidelines also stipulate that donor dogs should undergo complete clinical examination and laboratory tests including hematological and biochemical profile and serological assays, using IFAT (immune fluorescence antibody test) or PCR (polymerase chain reaction) for *Leishmania infantum*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Rickettsia rickettsii*, and *Babesia canis* (Italian Ministry of Health 2016). Preventive measures are indispensable in order to avoid infection in individual dogs, parasites spread by sick dogs, and re-infection of infected dogs (EFSA AHAW 2015; Abdolali et al. 2009; Maroli et al. 2010, and Mancianti and Meciani 1988).

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It is now generally accepted that resistance to CanL is primarily dependent on a dog's capacity to develop an appropriate predominant Th1 cell-mediated immune response in an overall balance between the Th1 and Th2 immunological profiles (Moreno et al. 2012, 2014; Jain and Jain 2015 and Starita et al. 2016).

In order to increase the prevention of Leishmaniasis in blood donors, vaccination could be used as an additional preventative measure. Several studies have demonstrated that vaccination with LiESP/QA-21 (CaniLeish®, Virbac) can stimulate a strong and long-lasting cell-mediated immunity, which is still present 1 year after the last dose of the primary vaccine course (Moreno et al. 2014; Martin et al. 2014; Oliva et al. 2014, and EMA 2011).

The aim of this study was to evaluate hematological, biochemical, serological, and molecular modifications in a group of healthy dogs participating in a voluntary blood donor program and receiving full coverage of immunization with CaniLeish®. This study provides new data to our previous investigation (Starita et al. 2016).

Materials and methods

Selection criteria The study took place between March 2015 and June 2016, continuing from the previous work that was performed from February 2013 to July 2014 (Starita et al. 2016). Upon written consent, 18 of the initial 27 client-owned dogs participating in a voluntary blood donor program at the Veterinary Transfusion Centre were included and six new dogs were added.

The following selection criteria were used to include dogs as blood donors: Dog Erythrocyte Antigen (DEA) 1 negative, absence of any clinical signs of disease, complete blood count values (CBC) (ProCyte Dx®, Idexx, Italy) and blood smear examination, serum biochemical profile (SBP), including total protein, albumin, urea, alkaline phosphatase, and alanine aminotransferase (Liasys®, Assel, Italy), and serum protein electrophoresis (SPE) in agarose gel (Pretty®, Interlab, Italy) within the reference ranges of the Veterinary Clinical Pathology Laboratory, Department Veterinary Sciences, University of Pisa. In addition, any dog with serological positivity titer for *Leishmania infantum*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* (IFAT, less than 1/40 titer) starting from 1:40 was excluded from the blood donor program. All dogs also received regular protection against ectoparasites, such as Effetix® Spot-On, a repellent based on the fipronil and cypermethrin formulation available on the market for topical use.

Screening of blood-donor dogs through speed Leish K™ assay Before starting the vaccination protocol with CaniLeish®, blood donor dogs were evaluated on the basis of criteria applied by the Veterinary Transfusion Centre previously reported in the selection criteria. All blood donor dogs, prior to administering CaniLeish®, were tested with the rapid test Speed Leish K™ (Virbac) to assess their serological negativity, as suggested by the manufacturer. Since the protective effect of CaniLeish® has not been studied in dogs already infected by *Leishmania infantum*, in the present study, any positivity showed by Speed Leish K™ was a reason for not administering the vaccine.

Vaccination of blood-donor dogs with CaniLeish® The lyophilized CaniLeish® vaccine was stored at + 4 / + 10 °C, reconstituted with 1 mL of its solvent, and administered subcutaneously in the withers region, followed by a gentle massage of the injection site. Dogs were monitored for about 30 min in order to observe the onset of possible reactions. The owners were advised to report to the authors any suspected reaction or possible adverse effect, in which case the dogs would need to be checked. The vaccine was administered according to the protocol indicated in the manufacturer's instructions: a first cycle of three inoculations, each one every 3 weeks, plus annual boosters. In the present study, for the second and third annual boosters, the vaccine was also administrated as a single injection.

Study design Twenty-four canine blood donors (14 females, 10 males; 9 Boxers, 7 mixed breeds, 3 Golden Retrievers, 2 Dobermanns, 1 Weimaraner, 1 Border Collie, and 1 Newfoundland) were included in this study.

As it was designed to continue the previous work of Starita et al. 2016, two Dobermanns, one Newfoundland, and three mixed dogs were added to the original group. Four Boxers and four mixed dogs were excluded because their owners had withdrawn from the blood-donor program.

The timing (*T*) of the blood sampling was as follows: T0 (basal, at the first immunization), T1 (at the second immunization), T2 (at the third immunization), T3 (2 months after the third immunization), T4 (at the first annual booster), T5 (at the second annual booster), and T6 (at the third annual booster). For some dogs, not all the biological data monitored at the different collection times were available.

In the present study, dogs were included as a single population sample even if they belonged to different subgroups according to their stage of immunization and blood collection. In fact, four subgroups were identified with the following collection times: subgroup 1 included five dogs at T0, T1, T2, T4, T5, and T6; subgroup 2 included eight dogs at T0, T1, T2, T3, T4, and T5; subgroup 3 included seven dogs at T0, T1, T2, T3, and T4; subgroup 4 included four dogs at T0, T1, T2, and T3.

Blood analysis used to monitor the canine blood donors The serum concentration of total protein and albumin and the SPE

were performed at the different collection times (T0-T6) whenever the blood samples were available (subgroup 1, T0-T6, 13 samples assayed; subgroup 2, T0-T5, 38 samples assayed; subgroup 3, T0-T4, 28 samples assayed; subgroup 4, T0-T3, 16 samples assayed). The serum CRP was assessed in: subgroup 1 at T5 and T6 (7 samples), subgroup 2 at T5 (8 samples), subgroup 3 at T3 and T4 (9 samples), and subgroup 4 at T3 (4 samples). The serological assays (IFAT *L. infantum*) were performed as follows: subgroup 1 at T0-T6, 22 samples tested, subgroup 2 at T0-T5, 40 samples tested; subgroup 3 at T0-T4, 28 samples tested; and subgroup 4 at T0-T3, 16 samples tested. The qlPCR were assayed in 26 samples as follows: subgroup 1 at T6 (5 samples), subgroup 2 at T5 (8 samples), subgroup 3 at T3 (2 samples) and T4 (7 samples), and subgroup 4 at T3 (4 samples). The extraction kit used for the qlPCR was the Blood/Cultured Cell Genomic DNA Extraction Mini Kit ® (Fisher Molecular Biology, Trevose, PA, USA), the primers used were L5.8S and LITSR for Leishmania (El Tai et al. 2000) and the thermocycler was the S1000™ Thermal Cycler (Bio-Rad Laboratories S.r.l., Segrate, Milan, Italy). The Speed Leish K™ was performed at T0 and at every annual booster, namely T4, T5, and T6 in all dogs.

Statistics Data distribution was assessed through the D'Agostino-Pearson test and had a non-parametric distribution. The Kruskal-Wallis test was performed for serum biochemical analytes, and SPE results obtained at different times, as reported above. For all tests, significance was set at $p < 0.05$. Statistical analysis was performed using MedCalc® Statistical Software (version 15.8, Ostend, Belgium).

Results

Serum biochemical analytes No statistical significance for serum total protein and albumin was found throughout the monitoring period (T0-T6) (data not shown). However, slight hyperproteinemia up to the maximum value of the reference range (5.8–7.8 g/dL) occurred in 29.5% (28/95) of the samples. The serum CRP comparison investigated at T3, T4, T5, and T6 was statistically significant (Table 1) with 53.6% (15/28) of samples with normal values and 46.4% (13/28) of samples with a slight increase outside of the reference ranges (0.0–0.3 mg/dL).

Serum protein electrophoresis Results from SPE are shown in Table 2 as percentage values and as absolute values (both using the median and 95% confidence interval). Our data showed a statistical significance ($p < 0.05$) of β-1-globulins (as absolute and percentage values) and β-2-globulins (as absolute and percentage values) at different collection times. The statistical significance of γ-globulins (as a percentage) was borderline ($p = 0.052$) and not detected at T4 and T6.

Table 1 C-reactive protein (CRP) data at a few collection times (*T*)

Analyte[a]	Time	Median	95% CI median
CRP (0.0–0.3 mg/dL)	T3	0.2*	0.1–0.3
	T4	0.2*	0.0–0.5
	T5	0.8*	0.2–1.3
	T6	0.8*	ND

Number of samples (*n*) analyzed at the different collection times of T3 $n = 6$; T4 $n = 7$; T5 $n = 10$; T6 $n = 5$

ND Not determinable due to the small number of samples

*Kruskal-Wallis test was $p < 0.05$

[a] Values in brackets are reference ranges

Serological tests The results of IFAT are reported in Fig. 1. Considering the whole monitoring time (T0-T6), the canine samples were negative in 73.3% (78/106) and positive in 26.7% (28/106) of samples. Out of the percentage of samples with positive IFAT titres, 9.5% (10/106) showed titres of 1/40, 8.5% (9/106) showed titres of 1/80, 6.7% (7/106) showed titres of 1/160, and 1.9% (2/106) showed titres of 1/320.

Molecular assay Samples for qlPCR were negative in 92.3% (24/26) of cases and positive in 7.7% (2/26) of cases. The only two positive samples (dogs) were recorded at T3 and T4 in subgroup 3. It should be noted that the same dog that was positive for qlPCR at T3, turned out negative at T4. The other dog that was positive for qlPCR showed an IFAT titer of 1/160. The concordance of negative results both for IFAT and qlPCR was 80.8% (21/26 samples), and the discordance of results from IFAT and qlPCR was 19.2% (5/26 samples). The discordance was for each of the five samples: negative for IFAT and positive for qlPCR, positive for IFAT at 1/160 titer and positive for qlPCR, positive for IFAT at 1/40 titer and negative for qlPCR, positive for IFAT at 1/80 titer and negative for qlPCR, and positive for IFAT at 1/320 and negative for qlPCR.

Discussion

Serum biochemical analytes and SPE The serum protein profile is considered to be one of the most reliable markers for the diagnosis and monitoring of canine Leishmaniasis. Total protein concentrations are markedly increased in sick dogs and can be over 10 g/dl. These increases are mainly due to high levels of β- and γ-globulins, due to the polyclonal lymphatic activation. The typical electrophoretic pattern is characterized by hyperproteinemia, hypoalbuminemia, polyclonal hypergammaglobulinemia, and the inversion of the albumin/

Table 2 Major serum proteins and electrophoresis fractions reported as percentage and absolute (in brackets) values collected at different times (T)

Analyte[a]	Time	Median % (g/dL)	95% CI median % (g/dL)
Albumin (47.5–58.5%) (2.6–4.1 g/dL)	T0	53.5 (3.5)	48.5–56.2 (3.3–3.7)
	T1	51.3 (3.6)	48.0–56.3 (3.3–3.9)
	T2	49.5 (3.3)	47.5–52.6 (3.1–3.5)
	T3	51.3 (3.6)	48.2–53.4 (3.2–3.8)
	T4	48.0 (3.4)	45.8–52.0 (3.2–3.6)
	T5	50.0 (3.8)	44.4–53.0 (3.3–4.1)
	T6	47.7 (3.3)	ND
α1-globulins (2.6–4.4%) (0.1–0.3 g/dL)	T0	3.8 (0.2)	3.2–4.0 (0.2–0.3)
	T1	3.3 (0.2)	2.9–3.8 (0.2–0.25)
	T2	3.3 (0.2)	3.0–3.9 (0.2–0.28)
	T3	3.3 (0.2)	2.9–3.5 (0.2–0.3)
	T4	3.4 (0.2)	3.1–3.9 (0.2–0.3)
	T5	3.4 (0.2)	3.1–4.8 (0.2–0.3)
	T6	3.2 (0.2)	ND
α2-globulins (10.7–18.0%) (0.6–1.4 g/dL)	T0	14.7 (1.0)	12.6–16.1 (0.8–1.1)
	T1	15.0 (1.1)	12.3–16.3 (0.9–1.2)
	T2	14.6 (1.0)	13.2–17.0 (1.0–1.1)
	T3	14.9 (1.1)	13.5–16.7 (0.9–1.2)
	T4	15.2 (1.0)	14.2–15.6 (1.0–1.2)
	T5	16.8 (1.3)	14.4–19.2 (1.0–1.5)
	T6	14.5 (1.0)	ND
ß1-globulins (5.9–13.3%) (0.3–1.0 g/dL)	T0	9.9* (0.6*)	8.7–11.7 (0.6–0.7)
	T1	6.7* (0.5*)	5.4–7.7 (0.4–0.5)
	T2	7.1* 0.5*)	6.1–7.5 (0.4–0.5)
	T3	7.9* 0.5*)	5.6–11.9 (0.3–0.8)
	T4	8.9* (0.6*)	5.7–13.7 (0.4–0.9)
	T5	9.7* (0.8*)	8.0–15.4 (0.5–1.1)
	T6	8.1 (0.6)	ND
ß2-globulins (7.2–14.4%) (0.4–1.1 g/dL)	T0	11.1* (0.7*)	9.0–11.6 (0.6–0.7)
	T1	14.3* (1.0*)	13.1–15.5 (0.8–1.1)
	T2	15.3* (1.0*)	13.9–15.6 (0.9–1.1)
	T3	12.6* (0.9*)	10.4–16.9 (0.7–1.2)
	T4	11.5* (0.8*)	11.0–15.3 (0.7–1.1)
	T5	10.3* (0.8*)	9.3–11.5 (0.6–0.9)
	T6	16.6* (1.3*)	ND
γ-globulins (6.6–11.6%) (0.4–0.9 g/dL)	T0	8.5* (0.5)	6.6–9.8 (0.5–0.7)
	T1	11.0* (0.8)	8.0–12.2 (0.5–0.9)
	T2	10.2* (0.7)	8.8–11.8 (0.6–0.7)
	T3	8.7* (0.6)	6.8–9.8 (0.5–0.7)
	T4	10.3 (0.7)	9.0–11.0 (0.6–0.8)
	T5	7.5* (0.6)	6.7–11.5 (0.4–0.8)
	T6	10.5 (0.8)	ND
Total protein (5.8–7.8 g/dl)	T0	7.1	6.7–7.7
	T1	7.6	6.8–9.1
	T2	7.4	6.6–8.6
	T3	7.4	6.8–10.0
	T4	7.2	6.9–7.3
	T5	7.7	7.0–8.5
	T6	7.6	ND

[a] Values in brackets are reference ranges. Number of samples (n) analyzed at the different collection times: T0 $n = 24$; T1 $n = 14$; T2 $n = 16$; T3 $n = 11$; T4 $n = 15$; T5 $n = 10$; T6 $n = 5$

ND Not determinable due to the small number of samples

*Kruskal-Wallis test was $p > 0.05$ for all time-checks except where the asterisk next to the median values either on percentage and/or absolute values is reported ($p < 0.05$)

[a] Values in brackets are reference ranges

globulin ratio. The drastic fall in albumin levels observed is generally due to renal or hepatic damage and to the inflammatory condition (Martinez-Subiela and Tecles 2002; Ciaramella and Corona 2003; Ciaramella et al. 1997).

Since our data did not show significant alterations in total protein concentration but a statistically significant modification in β1, β2, and γ-globulins at all collection times, a post-vaccination immunity may have developed. In addition, these globulin fractions showed a slight increase, which is not typical of the sick dogs affected by Leishmaniasis (Moreno et al. 2012; Martin et al. 2014; Oliva et al. 2014; EMA 2011; Bongiorno et al. 2013 and MorenoVouldoukis et al. 2014).

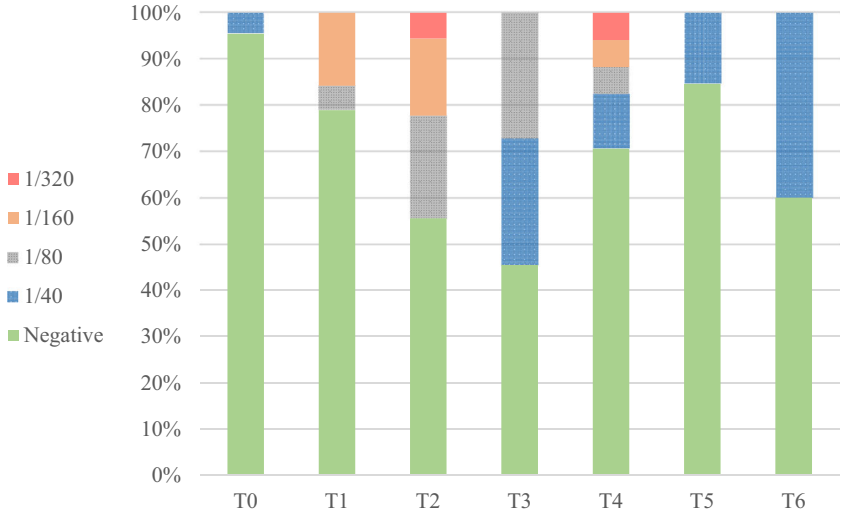

Fig. 1 IFAT titres for *L.infantum* collected at different times (*T*) collections

The investigation done with CRP showed a statistically significant modification at the few collection times, above all in the samples collected at the annual booster. This seems to be related to an active acute phase inflammatory response; however, a high rate of false-positive results is expected since there are many other conditions which show an increase in the acute phase proteins, such as surgery, steroid treatments, infectious and parasitic diseases, and other inflammatory processes (Martinez-Subiela and Tecles 2002).

Serological tests Before starting the vaccination protocol with CaniLeish®, the serological negativity of blood donor dogs for Leishmaniasis was evaluated prior to administering the vaccine using the rapid test Speed Leish K ™ (Virbac, Italy), as suggested by the manufacturer. Speed Leish K™ detects specific antibodies against the kinesins of *Leishmania*, which are not present in the excreted-secreted proteins (ESP) of the CaniLeish® vaccine (Ferroglio et al. 2013 and Rotondi 2013). However, Solano-Gallego et al. reported that the qualitative rapid tests showed a low sensitivity in subclinically infected dogs. Thus, these false negative results may lead to the vaccination of seropositive dogs, and could be a complicating factor in the evaluation of the vaccine efficacy in the field (Starita et al. 2016; Solano-Gallego et al. 2014, 2017).

In any case, in our study, all blood donor dogs were negative with IFAT titres at T0 except one seropositive dog, which had a starting titer of 1:40. This dog was included in subgroup 4 and received the first year of immunizations. This canine blood donor was negative at T2 and T3 and again positive at 1:80 titer at T4. No other modifications in clinical or clinico-pathological signs were noted and were related to Leishmaniasis infection or disease throughout the entire observation period. Concerning the IFAT results, our data show that the percentage of positive IFAT titers increased from T0 to T3, decreased again to T5, and lastly underwent a further increase from T5 up to T6.

In agreement with many previous studies (Sagols et al. 2012, 2013), our data showed an increase in the IFAT titer subsequent to vaccination (T0-T3) up to the seroconversion and then a gradual decrease. The trend in humoral immune response after the first annual booster (T4-T6), which was shown to be similar to the transient profile after the first cycle of vaccination, also matched results obtained in previous studies (Starita et al. 2016). It should be noted that a few dogs from the study by Starita et al. were included in the present study and belonged to dogs receiving the annual booster.

Serological studies with IFAT are however, characterized by a high coefficient of inter-and intra-assay variation. Furthermore, serological tests at low titres are non-specific and do not distinguish antibodies due to infection from those subsequent to immunization (Starita et al. 2016; Ferroglio et al. 2013; Rotondi 2013).

The IFAT results should thus be interpreted with caution in vaccinated dogs. Standard guidelines suggest considering as high only titres exceeding at least a fourfold difference with the threshold value of positivity for the reference laboratory and indicating infected or diseased animals (Starita et al. 2016; Paltrinieri et al. 2010). Although in our study, 6.7% of blood donor dogs showed titres of 1/160, and 1.9% showed titres of 1/320, these values should not be considered as evidence of infection based on the standard guidelines, and the increase in IFAT titres that we found is more likely due to the humoral immune response developed after the vaccination. On the other hand, other clinical and/or clinico-pathological evidence supports the diagnosis of infection by Leishmania. Regarding canine blood donors, it is advisable to look at any other evidence available or to add further diagnostic tests such as molecular investigation by PCR or clinico-pathological assays such as urinalysis. In addition, the blood bag should remain in the blood bank fridge until the completion of the additional diagnostic plan in order to prevent any transmission of *Leishmania spp.* by blood transfusion.

Molecular test The most useful diagnostic approach for the investigation of Leishmaniasis includes both the detection of specific antibodies against *L. infantum* through serological assays and evidence of the parasite DNA through molecular techniques (EFSA AHAW 2015; Maroli et al. 2010; Starita et al. 2016 and Solano-Gallego et al. 2011). A qualitative qlPCR was therefore provided in our study. The canine samples showed negative qlPCR in 92.3% (22/24) of cases and positive qlPCR in 7.7% (2/24). Unfortunately, qlPCR was available only for a few subgroups at a few collection times.

The concordance between the qlPCR results and IFAT titres was evaluated. The data showed 80.8% of concordance in samples between the negative results of qlPCR and IFAT titres, whereas 19.2% of samples showed discordance in the qlPCR results and IFAT titres. Among the discordance data, only two qlPCR positive samples were found, one sample was positive with IFAT titer at 1/160, and the other sample was negative with IFAT titer. These two blood donor dogs that showed a positive qlPCR were then thoroughly analyzed, and no evidence of clinico-pathological results (data not shown) typical of infected or sick individuals was found, i.e., characterized by polyclonal gammopathy and/or β-γ bridging at SPE (Solano-Gallego et al. 2011).

Studies evaluating PCR from different tissues of infected dogs however, have shown variable results, which could be explained by the heterogeneous distribution of the parasites in each tissue, the tropism of the Leishmania strain, and the local immune response (Martin et al. 2014 and Noli and Saridomichelakis 2014). The selection of the site sample for the PCR assay is thus very important. Lymph node biopsy has a high sensitivity, but sampling may be not easy without lymphoadenomegaly. Bone marrow aspirates are an appropriate alternative, but collection is invasive. Instead, the sensitivity obtained from blood samples is lower than with other tissues (Belinchón-Lorenzo et al. 2012).

However, the detection of the DNA of Leishmania does not always represent clinical disease, but may also show the presence of unviable organisms. The transfusion-transmission of Leishmania requires amastigotes to be present and alive in the blood of the donor, which need to survive storage in the blood bank. Previous reports have documented that Leishmania can be transmitted by whole blood, mononuclear cell, or plasma transfusion (Tabar et al. 2008).

As previously mentioned, the low sensitivity of the qualitative rapid test (Speed Leish K™) in infected subclinical dogs, may result in the vaccination of seropositive dogs and could represent a complicating factor for the evaluation of the vaccine efficacy in the field (Starita et al. 2016; Solano-Gallego et al. 2014). Since there is no sensitive test to discriminate antibodies against vaccination and natural infection with *L. infantum*, the use of quantitative serology as the only diagnostic technique for the detection of CanL in vaccinated dogs is not recommended (Solano-Gallego et al. 2017).

The limitations of our study were the low number of canine samples included that were not collected at each observation time, the different vaccination stages for the dogs included, and the lack of quantitative real-time PCR to assess the parasite load.

Conclusions

In this study, a group of healthy blood donor dogs were monitored over time in order to detect possible post-vaccination abnormalities. Our data show that after immunization with CaniLeish®, only minimal modifications in total protein concentrations, some globulin fractions (particularly beta and gamma fractions) and a mild increase in IFAT titres, and some occasional positivity in qlPCR for Leishmania may occur.

The significance of most of these results is likely due to the development of post-vaccination immunity. The positivity of qlPCR in blood samples should be investigated further by adding new sampling sites such as conjunctival scraping, lymphonode fine needle biopsy, and bone marrow aspirates. Dogs with positive qlPCR should be temporarily excluded from the blood donor group up to the end of further clinico-pathological investigations in order to ascertain the status of the infection. In conclusion, the use of CaniLeish® could be an important additional practice for preventive medicine along with topically measures, especially in those animals used for canine transfusion medicine.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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